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Algal Green Energy – R & D and technological perspectives for biodiesel production

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ABSTRACT

Energy is the utmost requirement for driving the organization and maintenance of entire ecosystem. Our continued dependency on fossil fuels such as coal, petroleum and natural gas as the prime source of energy has led to serious concerns about the future energy supply and security. Furthermore, over-consumption of carbon-based fossil energy sources raises serious environmental issues of global warming and climate change. To overcome the global energy demand and to enable economic as well as ecological development in a sustainable manner, technological progress for the utilization of renewable natural energy are essential to protect the environment and save energy in today's increasingly competitive world. To this end, algal biofuels are being claimed as an apt alternative energy source and in recent past, several taxonomic groups of algae have been studied and reported as an alternative to fossil fuels. It is envisaged that algal biomass could be readily processed into the raw material to make cost-effective biofuels and is being explored as an emergent and renewable green energy crops for the production of biofuels, especially biodiesel. Development of astonishing technological innovations in the field of algal genetic engineering has triggered remarkable output across the global energy sector for better biofuels. Several new techniques are being adopted for large-scale farming of microalgae intended for biofuel production. However, there are certain constraints for commercial-scale energy production from algae. The present review discusses the technological development and current information on the cultivation and process of biodiesel production form algae. Also, discussed are the technological development and genomic insights into the algal biomass and triacylglycerol accumulation for enhanced biodiesel production.

1. Introduction

Since ancient times, fossil fuels have been used as an efficient and ideal source of energy. In the last few decades, urbanization and industrialization has led to substantial extraction and consumption of fossil fuels such as coal, petroleum and natural gas for various purposes. Moreover, fossil fuels are carbon-based energy sources which pose serious environmental health issues due to emission of incredible amount of gaseous pollutants such as carbon dioxide (CO_2), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), carbon monoxide (CO), etc., upon combustion [1]. The harmful environmental impacts and global crisis of fossil fuels due to their over-consumption, and limited source in the nature has seriously impelled worldwide interest in search of alternative renewable energy sources [2–4]. Besides various natural energy sources, such as solar/wind power and hydroelectricity, different types of biomass are being used in various energy sector for power generation to meet the global fuel demand [5]. Conversion of renewable biomass to biofuels could be exceedingly feasible from ecological as well as economical view point, as fossil fuels are progressively becoming scarce and costly.

Algae including cyanobacteria (or blue-green algae) are major photosynthetic primary producers, maintaining the trophic energy dynamics and display a substantial role in sustainable development of aquatic or terrestrial ecosystems. Existence of different algae and cyanobacteria in diverse ecological niches even under adverse growth conditions compelled them to synthesize a range of valuable secondary biomolecules such as lipids or oils, carbohydrates, proteins, and several other feedstocks that can be used in production of biofuels and other co-

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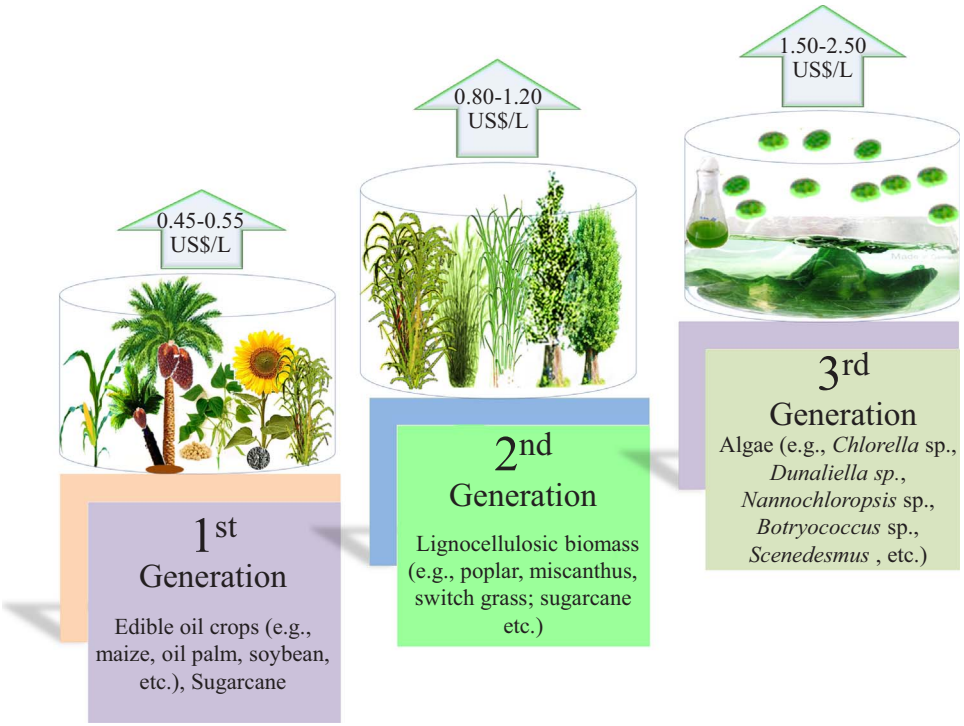


Fig. 1. The utilization of various natural resources for the production of first, second and third generation biofuels as the suitable alternatives to exhausting fossil fuels. As shown in Fig. 1, the production of biofuels from algae is quite higher than 1st or 2nd generation crops; however, the current cost of algal biofuel is comparatively higher (shown by arrow) (for details see [11,12].

products [6–10]. Algae can be employed as a very promising renewable energy sources due to their inherent capacity to fix the atmospheric CO_2 by the light-driven phenomena photosynthesis [11]. Moreover, algae can replace the first-generation feedstock such as edible crops (due to their food values), and second-generation lignocellulosic energy crops for the production of biofuels (Fig. 1) [6].

The net algal biomass yield per unit area is often higher than those of higher energy crops [12]. It has been shown that about 13.1 kg dry weight m^{-2} (over seven months) of brown algae was obtained under cultured conditions compared to about 6.1–9.5 kg fresh weight $m^{-2}yr^{-1}$ for sugarcane [13]. In comparison to higher energy crop, algae could be a potent biofuel feedstock due to their fast growth rate and per hectare productivity, cultivation on unproductive bare land using fresh/saltwater along with nutrients from waste water, and low cost downstream processing [14,15]. It has been speculated that around 200 barrels of oil can be achieved by growing the photosynthetic algae per hectare of land. The above estimated yield from algae is supposed to be several hundred times higher than that of a commonly used energy (biodiesel) crop (soybean) [7] (Fig. 2).

Usually, microalgae have far more extensively been used as a lipid-based biorefinery for biodiesel production than macroalgae due to their higher per hectare yield (158 t) than those of later 60–100 t) [14]. However, macroalgae offer ease of harvesting and comprising high content of carbohydrates, which can be processed into bioethanol. Macroalgae can produce a net energy of 11,000 MJ/t dry biomass compared to 9500 MJ/t by microalgae by gasification [16]. Algal biomass can be converted into different forms of biofuels such as bioethanol, biodiesel, bio-oil, and biogas (e.g., biohydrogen and biomethane) using different conversion techniques such as fermentation, transesterification, liquefaction, anaerobic digestion, and pyrolysis [6,17]. Some other strategies, including heterogeneous catalytic processes could also be employed for the transformation of algal biomass. Several catalysts are used for the conversion of various biomass-derived molecules such as levulinic acid [18] and/or gamma-valerolactone [19] for the production of value-added chemicals and transportation fuels. Recently, Yan et al. [20] have reviewed the use of layered double hydroxides (LDH) and its derived metal oxides (DMO) catalysts for the conversion of biomass-derived molecules.

Biofuel production from algae, including cyanobacteria is at developing stage and needs efficient and economically viable technological solutions for commercial-scale processes. Several pilot projects have been established worldwide to facilitate the mass production of algae and algal biofuels. Besides open pond cultivation, different kinds of photobioreactors have been explored for maximum algae production [21]. Development in genetic engineering has resulted into genetically engineered algae as a potent biofuel feedstock. However, there are several issues regarding the algal farming for biofuels, which needs to be addressed to advance the algae-based biofuel industry at commercial scale. There are concerns that algal farming at large-scale may cause exposure of harmful phycotoxins to both aquatic and terrestrial ecosystems [22,23]. Production of different algal toxins and other allergens or carcinogens may pose a serious threat to global public health as well as fundamental ecological processes due to their potential carcinogenicity. Thus, the safety concerns must be addressed before their production at commercial scale, in particular for genetically modified cultures.

2. Algal biomass: production and processing

Algae are ubiquitous in fresh or marine water habitats and are major biomass producers both in aquatic and terrestrial ecosystems. They exist in unicellular or large multicellular forms. Since ancient times, algae serve as potent multipurpose cellular factories for high-value products [24]. Besides the immense source of several valuable natural products, some species of algae are considered an excellent source of biofuels as a renewable source of green energy [25]. To reach a maximum output of any products, fast growth rate and high production of biomass is crucially required. Light, CO_2 and water are the prime factors for efficient growth of algae, including cyanobacteria; however, several other parameters such as temperature, pH, aeration, and nutrient availability also affect their growth and development. To compete with fossil fuels, cost-effective cultivation of algae at large scale is

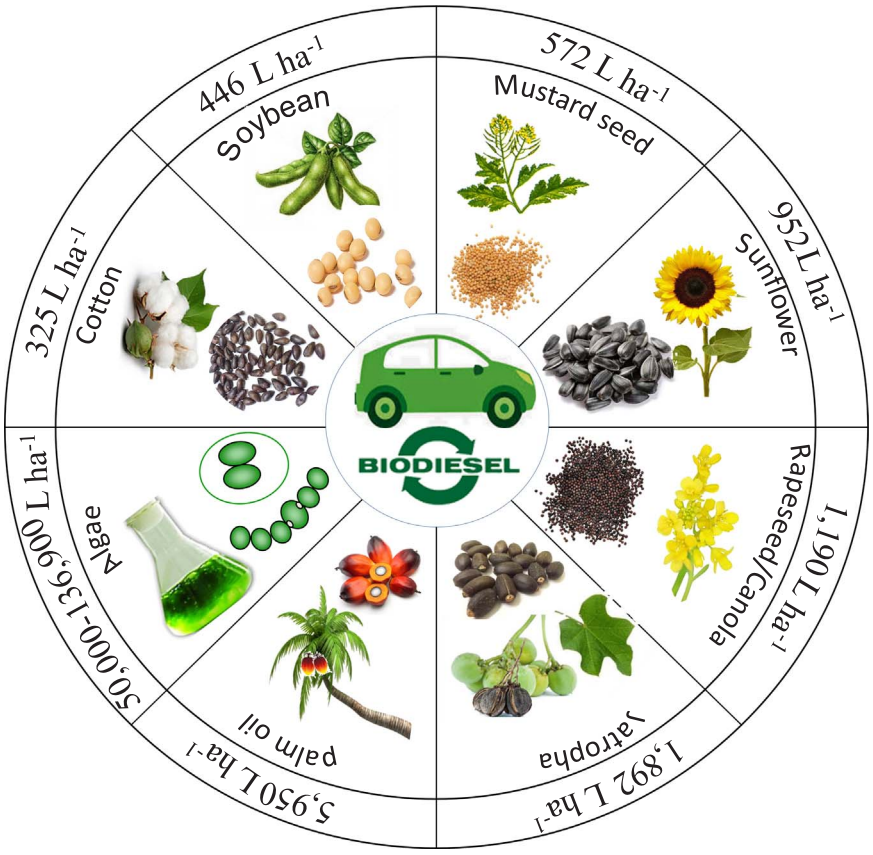


Fig. 2. The efficiency of different crop plants for biodiesel production (for details see [14,78,170]).

requisite. Energy-efficient methods for algae farming, harvesting and biomass processing are necessary for cost-effective production of biofuels from algae as sustainable alternative to fossil fuels [26,27].

Several models have been developed for large-scale production and processing of algal biomass for biofuels. In general production process, microalgae are primarily grown either in an open pond or in a closed photobioreactor (PBR) [28]. Different strains of algae are grown at commercial scale under open or closed cultivation systems using the various types of photo-bioreactors [29,30]. However, some parameters such as light source and their utilization, temperature control, availability of nutrients, maintenance of axenic culture, scale up cost, etc. are taken in to consideration for various large-scale culture systems to achieve the commercial success.

2.1. Open pond cultivation

The open pond systems include natural water reservoir as well as artificial large shallow ponds, tanks, circular stirred and paddle-wheel raceway ponds. Raceway ponds with two or more circulating channels and having a typical fluid depth of 20–40 cm are widely used as a large-scale algae cultivation system [31]. The structural design of a raceway pond has been improved for low cost production and process. Recently, computational fluid dynamics (CFD) technology has progressively been used to configure the raceway pond. It has been shown that the new designed raceway pond with sloping baffles and flow deflector can reduce the energy loss and apparently eliminates the dead zones [32]. The newly designed raceway pond inserted with propeller can consume around 60% less power than the traditional raceway pond, and can uphold the flow velocity, providing better mixing for photoautotrophic growth of microalgae [33,34].

Cultivation of microalgae under open pond system is comparatively easy to handle and maintain than those of closed cultivation systems. The main advantages of open pond cultivation system are the use of direct sunlight as the source of light energy, and low construction as well as operating costs [29]. However, there are certain limitations in operating the open pond system such as uneven distribution of solar light intensity and their utilization by the algal cells, continuous energy supplies for paddlewheels and water circulation, limited atmospheric CO_2 diffusion, maintenance of sufficient water depth, huge amount of water and nutrient input, ionic variation due to considerable water evaporation, low biomass productivity, and high risk of culture contamination.

Besides the use of simple raceway ponds, outdoor thin-layer high density culture system is also used for industrial-scale microalgae cultivation [35–38]. This system is consisted with an inclined surface exposed to solar-light where the algal suspension flows by gravity [38]. To overcome the problems associated with the installation of the baffles, arduous surface cleaning and some other difficulties, a modified thin-layer culture system was developed with reduction of surface inclination from 3.0% to 1.7% and in elongation of meandric arranged culture area [37]. The thin-layer culture system has been used to achieve the higher productivity of some green algae such as *Chlorella vulgaris* [39] and *Chlorella sorokiniana* [40] for biofuel and starch production, respectively. More than 50 g L^{-1} cell density can be obtained using the thin layer cultures system [37]. A biofilm-based algae cultivation system has been reported which is capable of producing direct algal biomass density of 96.4 kg/m^3; more than 35-times concentrated than the largest reported direct harvest, making the downstream process integration easier and less energy intensive. However, this system is under further technological improvement for achieving energy and water efficient algae cultivation targeted for biofuel production [35].

Open ponds are considered as an excellent habitat for a variety of

algae; however, culture contamination by other organisms and population crashes is one of the major primary challenge [2]. To avoid the contamination problem, the thin layer culture systems may be placed inside a glass house, enabling the high algal productivity even under unfavorable climatic conditions [37]. Some strategies such as upholding an extreme culture environment, for example, high salinity, alkalinity, or nutrient conditions have been adapted to overcome the influence of contamination. Several species of microalgae such as *Chlorella* sp., *Spirulina* sp. and *Dunaliella salina* are highly specific to certain extreme environments such as nutrient-rich media, high pH and bicarbonate concentration, and high salinity, respectively, and can be cultivated under open pond system with somewhat free of contamination by other species/strains of algae and other fast growing heterotrophs or predators [36]. Open pond system has not yet been fully optimized for the cultivation of some efficient oil producing algal species such as *Scenedesmus* sp., *Chlorococcum* sp. and *Tetraselmis* sp. [41]. Some chemicals could be used to mitigate the contamination problem in open pond system [42]. However, chemical strategies have not been fully effective against contamination at commercial-scale in open pond cultivation systems and efforts continue in exploring the chemical strategies to manage the contaminations in open pond algal cultivation systems ensuring the purity and stability of biomass [43]. Only a small number of algae can be grown successfully under highly selective environments in open pond cultivation systems, otherwise they are preferred to grow in closed cultivation systems either photoautotrophically, mixotrophically or heterotrophically. Compared with phototrophic and heterotrophic cultivation, mixotrophic cultivation is rarely used for biofuel production [44]. Overall, the proper management of contamination is indispensable in low-cost open pond system for large-scale algae cultivation.

2.2. Closed cultivation system-photobioreactors (PBR)

The closed cultivation systems are exceedingly expensive and difficult to scale up. Most of the closed systems are established indoors and need simulated light energy for photosynthesis, resulting in high energy costs. The conception of closed cultivation systems for large-scale microalgae production started very earlier [45]. Several modifications have been made to improve the basic designs and setup of closed photobioreactors for large scale algae production under controlled environmental conditions [46–48]. A wide range of closed cultivation systems such as big-bag or plastic foil bioreactors, flat-plate reactor, tubular serpentine type reactor and helical or biocoil type photobioreactor, manifold photobioreactors, stirred tank reactors, airlift bioreactors, floating photobioreactors, etc. have been developed for mass cultivation of microalgae or cyanobacteria [21,29,47,49]. Recently, self-cleaning mechanism has been introduced into different photobioreactors for self-cleaning of the tubes and reduced fouling [47].

The flat and tubular photobioreactors ensure reduced or no contamination, proper aeration and CO_2 supply, reduced evaporation and optimum light as well as temperature availability to the cells for higher biomass production. Contrary to open cultivation systems where loss of imported CO_2 is high, closed cultivation systems are important to maintain the CO_2 level for optimal cultivation of different microalgal spp. [47]. Proper mixing is essential to ensure the uniform distribution of light energy in a photobioreactor. It has been shown that regulation of light and dark cycles can improve the photosynthetic efficiency of algae growing in photobioreactors [48]. The closed photobioreactors can be operated in continuous culture mode under variable controlled climatic conditions for mass cultivation of a wider range of algal species.

To harp the advantages of open pond type system and closed photobioreactors as well as to overcome the certain limitations of the established photobioreactors, hybrid type of bioreactors comprising the characteristics of both of these have been devised [46]. A simple hybrid bioreactor is characterized by a covered open pond with gaseous space above the culture channel from the surrounding environment to prevent inflow and contamination from outer environment. Some other cultivation system such as floating photobioreactor made of polyethylene bags floating on the surface of water bodies has been developed owing to the possibility to use such a system on water surfaces especially at open-ocean surface without occupying the land area of coastal cities [50]. Table 1 shows various types of open and closed photobioreactors devised with their own advantages and disadvantages. None of these could be claimed to satisfy all of the norms of perfect PBRs [47]. Nevertheless, closed photobioreactors are consistently used to get contamination-free quality product with quite higher cell densities and reduced harvesting cost.

2.3. Integrated algae cultivation systems

To overcome the setup or production costs of algal biofuels, some advanced technology has been devised to scale up the cost effective production of biomass for biofuels and co-products (Fig. 3). Algae farming at commercial scale using wastewater and carbon sequestration have focused on reducing the cost of biomass production in conjunction with wastewater treatment and diminishing the global effects of greenhouse gas such as CO_2 [51].

2.3.1. Algal growth in waste water

Algae play a major role in aerobic treatment of municipal or industrial wastewater in the secondary treatment process for the removal of nutrients. Combining the microalgae cultivation with wastewater treatment system is considered one of the most promising ways to produce algal biofuel and other co-products in a cost-effective and ecologically sociable manner [52–56] (Fig. 3). Algae cultivation for biofuels production combined with wastewater treatment has several advantages. Wastewater may be an adequate and cheap source of several nutrients such as carbon, nitrogen and phosphorus required for enriched algal growth. It has been estimated that the addition of nutrient, water and CO_2 in algae cultivation at commercial-scale accounts for 10–30% of the total production costs. A combination of the processes could make nutrient and water addition preventable, consequently decreasing the biofuel production costs [54]. Undoubtedly, use of wastewaters has great potential towards algal biomass for biofuel production [52,57,58]. However, there are certain concerns regarding the use of wastewater, such as variation of wastewater composition, improper nutrient ratios, low light transmittance due to high turbidity and the presence of biotic or abiotic contaminants (e.g., heavy metals) that may hamper the algal growth and their cellular biosynthesis process. An extensive research disabling the contamination problems along with development of microalgae cultivation technology may be highly promising towards the low cost wastewater algae farming.

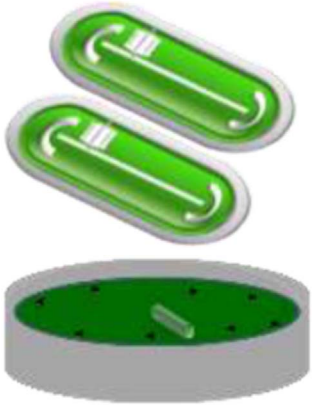
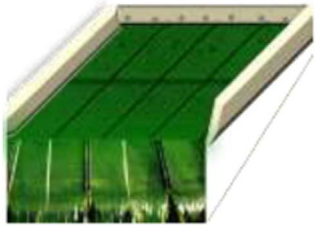
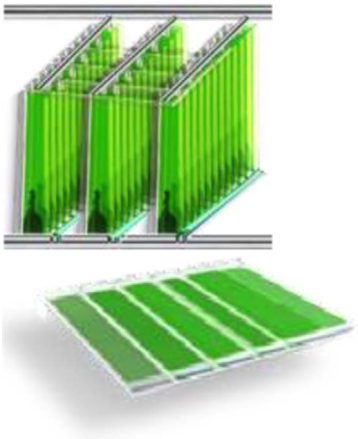
2.3.2. Photosynthetic carbon sequestration and algae cultivation

The production of phototrophic biomass for bioenergy and co-products predominantly instigated from the photosynthetic fixation of carbon dioxide [59]. In recent years, use of biofuels as a process of reducing the greenhouse gas such as CO_2 has received renewed impetus [60,61]. Microalgae use atmospheric CO_2 to produce biomass. Several pilot programs have found that algae can absorb as much as 85% of CO_2 content, minimizing the gas emissions from power plants. It has been supposed that a microalgae CO_2 sequestering facility with an area of around 10,000 ha comprising of 4000–5000 basic units for microalgae cultivation could exclude all of the CO_2 produced by a single 550 MW power station burning fossil fuel [62]. The immense potential of algae for CO_2-sequestration with increased biomass production has aroused

enormous concern towards their global cultivation strategies [63,64]. The use of flue gases as a source of CO_2 for algae growth may have prodigious positive economic effects, as it can minimize up to 90% costs associated with the growth stage, which represent the greatest costs and the investment and operating costs of the remaining stages [64].

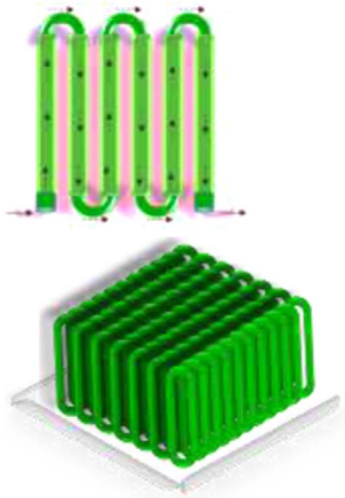
Some oleaginous algal species are capable to convert CO_2 from industrial flue gases or wastewater treatment plants into raw materials such as lipids and carbohydrates for production of 3rd or 4th generation biofuels, and hence they may be commercially viable alternative feedstocks to provide a solution for clean energy after the crucial exhaustion of fossil fuels [3,14]. An integrated microalgae cultivation system incorporating the wastewater and CO_2 emissions can be established in a marine environment for the production of algal biofuels. Large scale algae cultivation program has been facilitated near coastal cities where wastewater is discharged, using offshore membrane enclosures for growing algae (OMEGA) [65]. The OMEGA system comprises of flexible plastic made floating PBRs, intended to grow freshwater algae using wastewater effluent as the growth medium. The offshore settlement of the OMEGA system reduces the use of land area and assists large-scale algae cultivation in the vicinity of wastewater and flue gas (CO_2) from outfalls and onshore amenities, respectively. Furthermore, flue gases from industry or biogenic biogas

Table 1
Advantages and disadvantages of open and closed (photobioreactors) cultivation systems.

Cultivation system	Advantages	Limitations
Open cultivation system		
(e.g., Paddle-wheel raceway pond; Circular stirred pond) 	– Low construction and operating costs – Use of non-agricultural or Wasteland – Easy to clean up – Direct utilization of solar energy – -Relatively low energy consumption – Easy to handle and maintenance – High algae biomass production* – * Inconsistent report encompasses	– Susceptible to weather conditions. – Requirement of largeland areas – Continuous energy requirement for water circulation – Lack of sufficient mixing – Uneven distribution of solar light – Poor light penetration and limited atmospheric CO_2 diffusion – Low biomass productivity* – High risks of culture contamination and population crashes – Strain specific cultivation – Temperature fluctuation in the culture media – Ionic variation of media due to extensive water evaporation
Open-air thin-layer culture system 	– Simple construction and relatively low set-up costs. – Unusually high lifetime of the culture units without any Substantial additional costs – Efficient utilization of solar light energy by algal cells – Optimalalgal harvesting density – Relatively low energy demand for culture mixing and harvesting – Lower water demand – Relatively economy and easier control of certain growth factors such as nutrients, pH, and CO_2 concentration	– Higher investment costs compared with the raceways ponds – Arduous and time consuming cleaning process of culture surface and retention tank – Species/strain specific culture – Loss or CO_2 by desorption into atmosphere – Chance of contamination – Susceptible to weather conditions such as heavy rains or high temperature in sunny summer – Difficulty in maintaining the optimal culture temperature – Possibility of photoinhibition of the algae in the upper layer exposed to high irradiance
Closed cultivation system (Photobioreactors)		
Vertical or horizontal Flat panel/plate photobioreactor 	– Suitable for outdoor mass cultures – Light illumination overlarge surface area – Maximum solar energy harvesting – Better photosynthetic efficiency – Low accumulation of dissolved oxygen – Low chance of culture contamination – High-density algal biomass production – Improved homogenization of the culture – Easy sterilization or clean up Process	– Low surface-to-volume ratio – Difficult to scale-up – Poor temperature control – Possibility of hydrodynamic stress – Some degree of wall growth

(continued on next page)

Table 1 (*continued*)

Cultivation system	Advantages	Limitations
Open cultivation system		
Tubular photobioreactor 	– Suitable for outdoor mass cultures – Low risk of photoinhibition – High mixing efficiency – Larger surface area to light/sunlight exposure – High surface-to-volume ratio – Relatively low shear stress on tubes with good biomass productivity – Minimum chance of culture contamination, allowing maintained monoalgal culture condition – High photosynthetic efficiency	– Possibility of cell damage due to shear forces generated by hydrodynamic stress of pumping – Chance of increased dissolved oxygen levels – Some degree of wall growth and fouling – Requires large space
Column (cylindrical) photobioreactor 	– Relatively low-cost, compact and easy to operate – Most efficient mixing – High volumetric mass transfer rates and the best growth conditions – Lowest losses of CO_2 – Low risk of photoinhibition – Low risk of contamination and bio-fouling – Low shear stress – Easy sterilization process – Relatively low energy Consumption – Easy to O_2 release	– Sophisticated in construction – Chance of shear stress – Reduced surface area illumination – Difficulty in efficient culture mixing for large-scale processes – Chance of cell damage due to the bursting of gas bubbles

plant could be an inexpensive source of CO_2 for large scale algae farming in closed cultivation and outdoor operated thin layer culture systems [66,67]. Besides CO_2, several nutrients like nitrogen, phosphate and other minerals can be made available from inexpensive natural sources such as agro-industrial waste water bodies as well as from residual algal biomass after hydrolysis in product isolation or from digestate in biogas plants [67]. Moreover, integration of waste management via microalgae CO_2 sequestration as a carbon source [68] and wastewater utilization systems [9,65] can be technologically advanced and instigated at global level for the production of algae based bioenergy and co-products (Fig. 3).

3. Harvesting of algal culture

The process of harvesting and dewatering is one of the most important and challenging steps towards sustainable economics of algae-based fuels production [69], since the process involves high operational costs because of the dilute nature of microalgal cultures and their small sizes. Efficient harvesting of cultivated algal biomass is prerequisite for mass production of biofuel precursors [9,70,71]. A particular harvesting method may not be appropriate to recover all forms of algal biomass, consequently screening and identification of an efficient process is extremely needed to harvest the algal culture as per their physico-chemical properties [72], since the harvesting cost may account approximately up to 20–30% of the total biomass production cost [73,74]. Moreover, efficient and cost-effective methods of algae harvesting from water is a major problem towards industrial scale processing due to the fact that eukaryotic microalgae, and cyanobacteria are small in size ranging from 3 to 30 μm [74] and 0.2–2 μm [75], respectively. Two-step separation is practiced to reduce the associated costs of algal harvesting, i.e., thickening and dewatering procedures, where microalgal slurry is concentrated up to 2–7% and 15–25%, respectively, of the total suspended solids of microalgal slurry [76]. Algal harvesting cost as assumed above must be refined and implemented over time to decline the production cost, which will boost the economic pull towards the production of algae based renewable energy. Some common methods such as physical (e.g., centrifugation, filtration, flotation, gravity sedimentation), chemical (e.g., flocculation), and biological (e.g., bio-flocculation) are used to harvest the different species/strains of algae as per need to obtain the desired end products [44,72,77].

3.1. Physical methods

3.1.1. Centrifugation

Centrifugation is the finest and single-step processes of harvesting the microalgal cells within a short period with a high harvest efficiency of ~ 95%; however, it depends on the algal species and is not suitable for all types of microalgae. Furthermore, the use of centrifuges becomes more problematic as high gravitational and shear forces can damage the cell, causing the loss of valuable constituents into the medium. The concentration of 100–200 g/L_{algae} can be achieved by the centrifugation of algal broth with 10–20 g/L_{algae} [78]. Moreover, processing a large amount of culture using centrifugation is rigorous and expensive with a

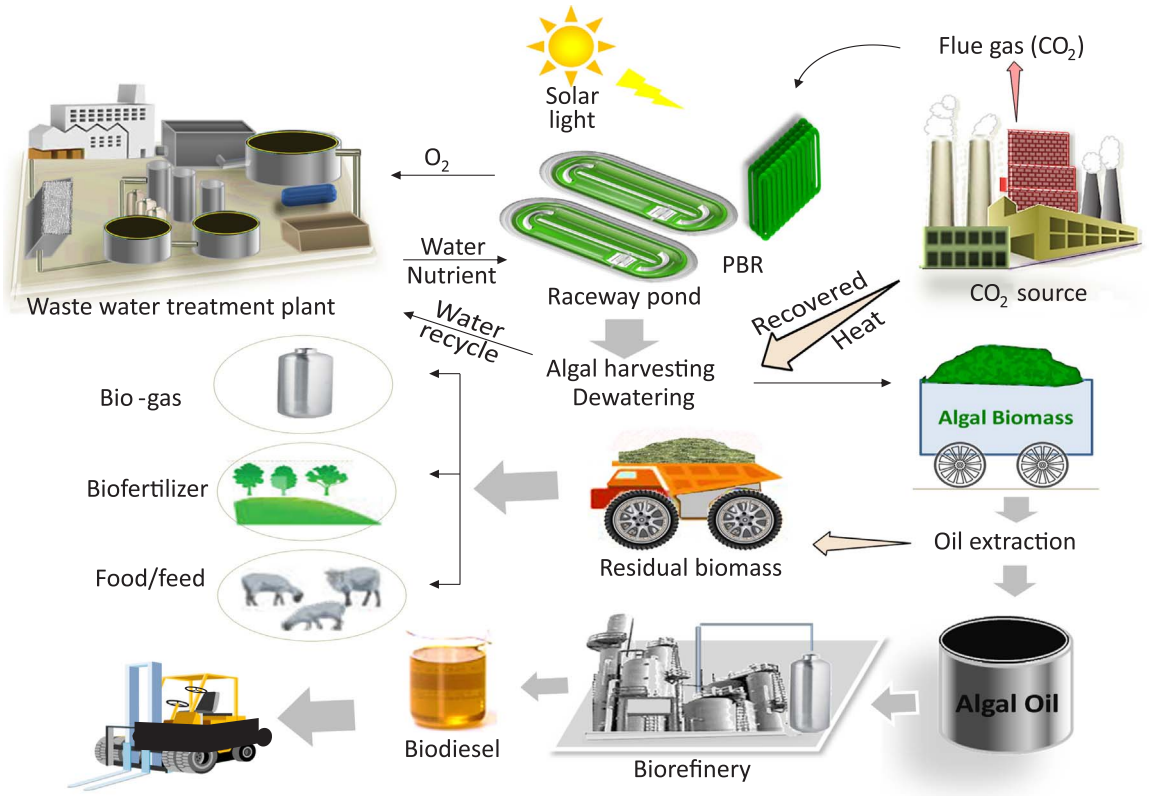


Fig. 3. A simplified overview of an integrated system involving wastewater integration and CO_2 sequestration for algae cultivation and biofuel production.

high energy demand [74,78]. The cost of energy inputs to the cultivation or harvesting processes often go beyond the energy content of the microalgal biomass [2,14]. The energy consumption assessment for harvesting microalgae by centrifugation was measured at 8 kW h/m^3 of microalgae suspension at a feed rate of 1 L min^{-1}. However, it has been verified that flow rates of 18 L min^{-1} can considerably reduce the harvesting costs up to 10-fold [79]. Therefore, large-scale algae harvesting by energy-intensive centrifugation for biofuel production is not justifiable and needs addition of some alternative methods.

3.1.2. Filtration

The process of filtration is carried out to collect the algal cells of very low density using different sorts of membranes with a particular pore size and a suction pump. It has been shown that the tangential flow filtration is a high rate (70–89%) method for harvesting the microalgae with preserved cellular structure [80]. However, there are various concerns with this harvesting process such as filter blockage, inefficient cell mass recovery, extensive washing requirements of screen filters, high maintenance costs and the requirement of large amounts of energy. Some changes in filtration design such as incorporation of reverse-flow vacuum and direct vacuum with a stirring blade above filter, incorporation of antifouling microfiltration, etc. have made this system economically feasible for a certain extent [80,81]. Usually, the filtration is a discrete process of harvesting after algae cultivation; however, it can be combined with the photobioreactor as a developing membrane photo-bioreactor (MPBR) system with some measures to control membrane fouling- a critical situation at a higher biomass concentration [82,83]. The process of ultrafiltration was effectively carried out for the first time to concentrate *Scenedesmus quadricauda* up to 15% in a single step batch filtration by applying air bubble scouring and backwashing to control membrane fouling [84]. Two step membrane filtrations for algae harvesting were also achieved, using polymeric and ceramic membranes at the primary and the secondary steps, respectively [85]. Readers interested in detailed information about the summary of studies on microalgae harvesting using various membrane technologies are referred to the review articles by Bilad et al. [86].

3.1.3. Gravity sedimentation

Gravity sedimentation is generally used to separate out different types of microalgal cells by impelled sedimentation velocity. Different algal cells have different settling velocity [87]. Effective harvesting of microalgae depends on the density of microalgal cells. The efficacy of this method is not reliable for routine harvesting due to very low microbial settling rates and worsening of the biomass during the settling time [2,9]. Moreover, this separation technique is widely used in wastewater treatment for cost-effective bulk removal of the solid particles. Lipid-rich microalgal cells are liable to become more buoyant and consequently less responsive to settle. Application of a suitable flocculants is advisable prior to gravity sedimentation to increase the rate of microalgal settling [44,70,72,74,88].

3.1.4. Flotation

Harvesting of microalgae by flotation is more advantageous and effective than sedimentation and other methods, owing to the low microalgal density and self-floating characteristics [89,90]. Some species/strains of microalgae such as *Anabaena*, *Microcystis*, *Spirulina* and *Nostoc* naturally float on the media surface due to the presence of gas vesicles; however, the efficiency of their flotation may also depend on their growth phase and hydrophobicity. The flotation of several algal species is induced by air bubbles from supersaturated water or ozone-rich air. This is a gravity separation process where air or gas bubbles attach to a solid particle by means of collision and move them up on liquid surface [91]. The attachment of a particle with a bubble depends on the possibility of adhesion once collision happened. The main advantage of flotation process is its large scale harvesting feasibility, low operational space requirements, comparatively short process times, and lower

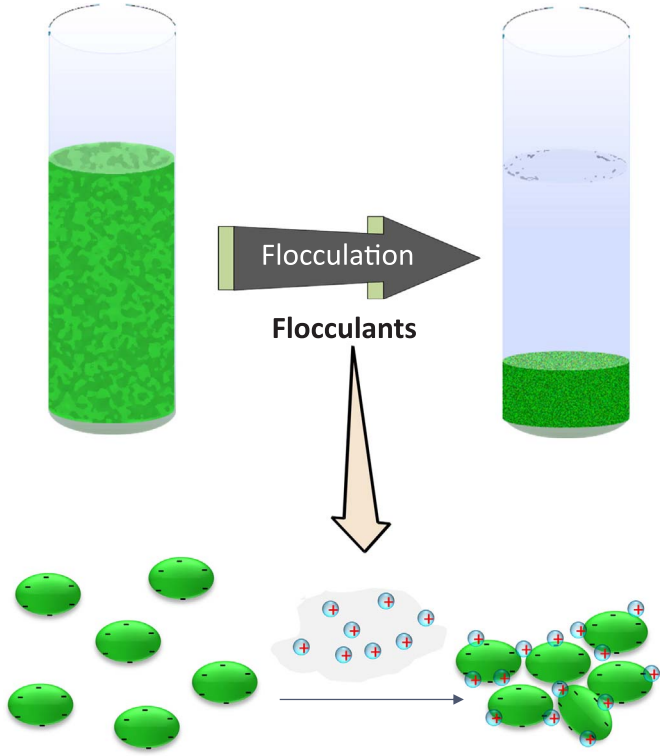


Fig. 4. Diagrammatic presentation of the flocculation process of microalgal cells based on charge neutralization by means of cationic flocculants.

initial equipment step up costs [89]. The process of flotation is mainly applicable for the separation of freshwater microalgae such as *C. vulgaris*, rather than marine microalgae, since salinity is a critical factor for bubble-cell adhesion, leading to decreased flotation efficiency [92]. The process of flotation at large scale often needs the use of certain surfactants to increase the extent of algal flotation on the water surface [89]. Moreover, the process of flotation is mainly accomplished by either dissolved air flotation (DAF) or dispersed flotation (DiAF), based on the size of bubbles [93]. Both dissolved air flotation and dispersed flotation generate bubbles ranging from 10 to 100 μm and 700–1500 μm in diameter, respectively [44,70,93].

3.1.4.1. Dissolved air flotation (DAF). In general, the supersaturated water in a pressure tank is released into a flotation tank at atmospheric pressure; where dissolved air precipitates out of the water forming small bubbles. The produced air bubbles adhere to the particles (microalgae) carrying them to the water surface at the top of the flotation tank [94–96]. The energy consumption associated with DAF was determined up to 7.6 kWh/m^3 due to the demand of high pressures for water supersaturation [89,94]. Several factors such as tank air pressure, particle size, particle floating rate, recycle rate and hydraulic retention time, etc. determine the harvesting of microalgae by DAF. This flotation process is often incurred by chemical flocculation. The cyanobacterium *Microcystis aeruginosa* was recovered with a maximum removal efficiency of 87%, when using a cationic surfactant into the saturator [97]. The residual surfactant can be removed via ozone treatment, followed by granular activated carbon [98]. The addition of cationic surfactants was reported to be beneficial, due to reduced costs on coagulant dosage. Coagulation with magnesium was found more effective for harvesting the microalga *Chlorella zofingiensis* with DAF [99]. DAF is supposed more commonly used method for large scale biomass harvesting [9,91], which produced lower turbidities [100] and remove microalgae more efficiently than dispersed air flotation (DiAF). However, based on merely limited parameters considered during different studies as reported above, it is tough to determine that DAF is an effective microalgae harvesting method. There are certain drawbacks associated with DAF, such as application of high dosage of flocculants, flocs breakage due to large bubble size and high energy consumption [70,94,101].

3.1.4.2. Dispersed air flotation (DiAF). In case of dispersed air flotation bubbles are formed by a high speed mechanical agitator and an air injection system. Contrary to DAF, dispersed air flotation is operated at 15 psi and energy consumption of 3 kWh/m^3 [94]. The surface hydrophobicity of the particle may greatly affect the possibility of microalgae-bubble attachment [102].

A combination of dispersed air flotation with the foam flotation system was optimized to recover the algal biomass [103]. Foam flotation seems to be a viable and attractive method for harvesting microalgae biomass [69,103,104]. A broad distribution of bubble sizes and rise velocity greatly affect the efficiency of a foam flotation microalgae harvesting process [105]. Certain surfactants or collectors such as cationic N-cetyl-N-N-N-trimethyl ammonium bromide (CTAB) are used to increase the hydrophobicity of the negatively charged cell for flotation by air bubbles or to encourage their surface accumulation [91,103–105]. Moreover, CTAB assisted foam flotation with the miniaturization of bubbles could be a cost-effective harvesting method which involves considerably lower rates of chemical dosage compared with dispersed air flotation. It was revealed that foam flotation consumes energy about 0.015 kWh/m^3, providing an economical technology for algae harvesting [103–105]. Some other methodologies such as electrolytic flotation [70], microflotation [89], ozone flotation [106,107], ballasted flotation [108] have also been optimized for algae harvesting. Overall, the process of flotation can be used to harvest the algae; however, a significant research is requisite to scale-up low energy harvesting techniques for the intended biorefinery system.

3.2. Chemical methods

3.2.1. Flocculation

The harvesting of microalgae by flocculation is considered an efficient method among the above mentioned methods, since recovery of a large quantity of microalgal cells of different taxonomic groups can be achieved by this process [93,109,110]. Flocculation is a process of aggregation of discrete particles (e.g., microalgae) or cells into larger particles by colliding and adhering to each other via the interaction of the flocculant with the surface charge of the cells (Fig. 4). Several flocculation methods, such as autoflocculation, bioflocculation, and chemical flocculation using polymeric coagulants or inorganic salts are used for algae harvesting. Moreover, the selection of flocculants depends on the final product and it is more important that it should be inexpensive, nontoxic and functional at low concentrations.

3.2.1.1. Autoflocculation. Autoflocculation is the cost-effective, nontoxic, simple process of settling of microalgae as a result of increased alkalinity (pH) due to an inconsistent situation of photosynthetic CO_2 consumption and increased precipitation of inorganic carbonate ions with algal cells [111,112]. Moreover, phototrophic cultivation of microalgae under limited CO_2 condition may result autoflocculation of algal cells. Experimentally, the process of autoflocculation can be shown by maintaining a definite pH value of the culture broth by the addition of an alkaline solution [111–113]. Overall, the supersaturation state of the culture medium due to excess precipitation of positively charged (calcium and phosphate) ions provides a solid surface to the negatively charged microalgal cells resulting in their accumulation at the bottom surface [93]. Even though, autoflocculation has great advantages over pre-concentration of microalgae [114], they are not used at commercial scale, as they may change the cell composition in the course of controlled flocculation [115]. Recently, an auto-floating system was developed in heterocysts of filamentous cyanobacteria

Table 2
Some synthetic and natural flocculants used in algae harvesting.

Flocculants	Type	Optimal dose mg/l	Optimal pH	Tested algal spp.	Cell conc.	Recovery rate/ time	References
Inorganic flocculants							
Ferric chloride ($FeCl_3{\cdot}6H_2O$)	Polyvalent cationic metal salts	122	> 6.0	*Chlorella*sp.	15 mg/L	93%, 75 min	[326]
Aluminum sulfate	Polyvalent cationic metal salts	41,077	6.8–7.0	*Chlorococcum* sp. R-AP13	na	87%, 12 min	[113]
Ferric chloride	Polyvalent cationic metal salts	11,350–12,970	6.8–7.0	*Chlorococcum* sp. R-AP13	na	92%, 12 min	[113]
Alum ($Al_2(SO_4)_3{\cdot}12H_2O$)	Polyvalent cationic metal salts	140	7.0	*Chlorella* sp.	30 mg/L	91%, 75 min	[326]
$FeCl_3$	Polyvalent cationic metal salts	150	na	*Scenedesmus* sp.	0.54 g/L	> 97%, 2 min	[327]
$Ca(OH)_2$	Cationic inorganic flocculant	400	na	*Scenedesmus* sp.	0.54 g/L	90%, 120 min	[327]
$Al_2(SO_4)_3$	Polyvalent cationic metal salts	100	5.0	*Scenedesmus* sp.	0.54 g/L	> 90%, 10 min	[327]
Alum	Polyvalent cationic metal salts	100	6.0	*Scenedesmus* sp.	0.54 g/L	> 90%, 10 min	[327]
Alum	Polyvalent cationic metal salts	10.8	na	*Nannochloropsissalina*	15–20 g/L	99%, 40 min	[328]
Polyaluminum chloride	Inorganic polymer	20	6.5–7.6	*Nannochloropsis gaditana*	132 mg/L	60%, 15 min	[329]
$Al_2(SO_4)_3$	Inorganic polyvalent cationic metal salts	1500	7.2	*Scenedesmus* sp.	0.4 g/L	> 99%, 12 min	[150]
Aluminum sulfate ($Al_2(SO_4)_3$	Inorganic polyvalent cationic metal salts	275	7.5	*Choricystisminor*	1.0 g/L	> 95%, 62 min	[145]
$Al_2(SO_4)_3$	Inorganic polyvalent cationic metal salts	100–300	6.4–11	*Chlamydomonas reinhardtii*	0.03–1.06 g/L	> 90%, 22 min	[120]
$Al_2(SO_4)_3$	Inorganic polyvalent cationic metal salts	100–300	6.4–11	*Scenedesmus* sp.	0.05–1.0 g/L	> 90%, 22 min	[120]
$Al_2(SO_4)_3$	Inorganic polyvalent cationic metal salts	100–300	6.4–11	*Schizochytrium limacinum*	0.93–4.65 g/L	> 90%, 22 min	[120]
Ferric chloride ($FeCl_3{\cdot}6H_2O$)	Inorganic polyvalent cationic metal salts	250	7.5	*Choricystisminor*	1.0 g/L	> 95%, 62 min	[145]
Polymeric/organic flocculants							
Superfloc C-492	Cationicpolyacrylamide	25	7.0	*Chlorella vulgaris GKV1*	20×10^6 cells/mL	~ 80%, 120 min	[123]
Sibfloc-718	Cationicpolyethylenoxide	15	7.0	*Chlorella vulgaris GKV1*	20×10^6 cells/mL	~ 80%, 120 min	[123]
Sibfloc-718 + $FeCl_3$	Inorganic polyvalent cationic metal salts + Cationicpolyethylenoxide	7.5 + 50	7.0	*Chlorella vulgaris GKV1*	20×10^6 cells/mL	90%, 60 min	[123]
Zetag 7557	Cationicpolyacrylamide polymer	20	na	*Neochloris oleoabundans*	*0.46 g/l*	88–99%, 2 h	[124]
Zetag 8185	High molecular weight polyacrylamide polymers	5	na	*C. vulgaris*	*260 mg/L*	100%, 60 min	[125]
Zetag 8819	Cationic polymer	34	7.0	*Chlorella*sp.	20 mg/L	98%, 75 min	[326]
Flopam FO 4990 SH	Polyacrylamide cationic polymers	0.55	na	*Nannochloropsis oculata*	290 mg/L	90%, 60 min	[125]
Flopam FO 4990 SH	Polyacrylamide cationic polymers	1.66	na	*Chlorella vulgaris*	260 mg/L	99%, 60 min	[125]
Flopam FO 4550 SH	Polyacrylamide cationic polymers	1.66	na	*Chlorellavulgaris*	260 mg/L	100%, 60 min	[125]
PK55H	Cationic polymer	1.5–4.0	na	*Chlorella* sp., *Scenedesmusacuminatus*	1×10^7 cells/ mL	< 95%, 60 min	[118]
KW100	Cationic polymer	25–35	na	*Chlorella* sp.; *Scenedesmus acuminatus; Chlamydomonas reinhardtii*	1×10^7 cells/ mL	90%, 60 min	[118]
Magnafloc LT25/27	Anionic polymer	0.1–0.5	10.2–10.6	*Chaetoceros calcitrans*	*na*	90–98%, 4 h	[330]
Greenfloc 120	Cationic starch	70	5–10	*Parachlorella* sp.	430 mg/L	> 90%, 1 h	[122]
Cargill C*Bond HR 35.849	Cationic starch	120	5–10	*Parachlorella* sp.	300 mg/L	> 90%, 1 h	[122]
EM16	Cationic Polyelectrolyte	10	8	*Muriellopsis* sp.	< 2.0 g/L	> 90%, 15 min	[119]
Inulin	Cationic biopolymer	60	7.4	*Botryococcus* sp.	na	> 88%, 15 min	[331]
Tanfloc SL	Cationic tannin polymer	5	na	*C. vulgaris*	260 mg/L	100%, 60 min	[125]
Tanfloc SL	Cationic tannin polymer	5	na	*N. oculata*	290 mg/L	97%, 60 min	[125]
Chitosan (natural)	Cationic polyelectrolyte (deacetylated polymer of chitin)	20	8.0	*Chaetoceros calcitrans*	na	> 90%, 4 h	[330]
Chitosan	Cationic polyelectrolyte	15	7.0	*Spirulina* sp., *Oscillatoria* sp. *and Chlorella*sp.	*na*	90%, 60 min	[130]
Chitosan	Cationic polyelectrolyte	20	9.9–10.0	*Phaeodactylum tricornutum*	104.62 mg/l	> 90%, 30 min	[332]
Chitosan	Cationic polyelectrolyte	200	7.5	*Euglena gracilis*	1.75×10^6 cells/mL	96–98%, 2 h	[333]
Chitosan	Cationic polyelectrolyte	120	6.0	*Chlorella vulgaris*	1 g/L	~ 99%, 3 min	[334]
Chitosan	organic cationic polymer	10	6.0	*Chlorella sorokiniana*	0.5–1.0 g/L	~ 99%, 45 min	[335]
AC-g-P(DMC-MACPPC)	Acryloyl chitosan Anchored copolymer	20	7.0	*Chlorella vulgaris*	*na*	70%, 60 min	[133]

(*continued on next page*)

Table 2 (*continued*)

Flocculants	Type	Optimal dose mg/l	Optimal pH	Tested algal spp.	Cell conc.	Recovery rate/ time	References
Chitosan + Fe3O4	Cationic polyelectrolyte + metal salts	1.6 (Chitosan) + 4 (Fe_3O_4)	7.0	*Microcystis aeruginosa*	4.8 × 10^6 cell/mL	99%, na	[336]
Zetag 7650 + Aluminum sulfate (AS)	Synthetic cationic polyelectrolytic polymer + inorganic cationic metal salts	10 (Z- 7650) + 50 (AS)	na	*Tetraselmis suecica*	0.42 g/L	~ 100%, 1 h	[80]
Polyacrylamide	Inorganic polymer	50	12.0	*Scenedesmus sp.*	0.54 g/L	60%, 10 min	[327]
Ecotan AR® (natural)	cationic polytannine coagulant	10	7.7	*Mixed Microalgal spp.*	*2.8 g/L*	> 90%, 10–20 min	[131]
Tanfloc SG ® (natural)	Cationic tannin-based natural flocculants	50	7.9	*Mixed Microalgal spp.*	*2.8 g/L*	> 90%, 10–20 min	[131]
poly (γ-glutamic acid) (γ-PGA)	Anionic biopolymer	~ 20.0	7.5	*Chlorella vulgaris*	*0.57 g/L*	90%, 2 h	[139]
poly (γ-glutamic acid) (γ-PGA)	Anionic biopolymer	~ 20.0	7.5	*Chlorella protothecoides*	*0.6 g/L*	> 95%, 2 h	[139]

using a photosynthesis inhibitor, diuron (1.0 mM), which inhibited O_2 production and resulted in a high rate of H_2 production in heterocysts. This auto-floating process recovered about 91.71% of the accumulated microalgal biomass from the liquid media [116].

3.2.1.2. Chemically induced flocculation. Harvesting of microalgal cells at large scale is also achieved with the application of certain chemical coagulant/flocculants such as organic/polymeric flocculants or inorganic flocculants. Certain chemicals (or flocculants) are used to induce flocculation, increasing the size of the cell aggregates to settle out of suspension. Naturally, flocculation leads to sedimentation in many older algal cultures, otherwise forced flocculation is required to promote sedimentation. A large number of either synthetic or natural chemical compounds such as alum, cellulose, chitosan, lime, cationic polymers praestol and polyacrylamide, aluminum sulfate, salts, and different surfactants are used in the flocculation process [25,80,93,117,118] (Table 2). Higher flocculation efficiencies were shown with polyelectrolytes than with metal salts [119,120]. Moreover, flocculation efficiency is dependent on several factors such as the coagulant type and charge as well as on the microalgae species [118–120].

3.2.1.2.1. Polymeric flocculants. Polymeric flocculants can either be cationic, anionic, or nonionic natural or synthetic molecules. The mechanisms behind polymeric flocculants used for harvesting of microalgae are based on the charge density, and chain length of a particular polymer meant for optimum particle bridging [121]. The efficacy of polymeric flocculation is determined by the extent of exposure of the microalgal surface by a particular polymer. The exposure of cell surface by the polymer beyond or below the optimum requirement may lead to either stalled or insufficient bridging, respectively. A number of parameters such as optimal dose, size and charge of polymeric flocculants, ionic strength or pH of the medium, surface charge density of the microalgal surface, chemical composition and concentration of the microalgal suspension, size of the microalgal flocs, functional groups on microalgal cell walls and extent of mixing affect the degree of effective polymeric flocculation process for algal harvesting [74,93]. High molecular weight bridging polymers and algal floc sizes in excess of 100 μm are considered effective for microalgal sedimentation [100]. The cationic flocculants are considered most effective for the recovery of freshwater and marine microalgae [80,117,119,122–125], whereas anionic or nonionic polymers have no or less efficacy to flocculate microalgae due to the fact of prevalent repulsion between charges or the insufficient distance to form adequate bridging links between particles. However, the cationic metal salt flocculants may adsorb irreversibly to the algal biomass and potentially interfere with solvent extraction of lipids from the biomass. The use of polymeric coagulants for flocculation of microalgae from saline environment is relatively ineffective due to shrinkage of polymers and failing in bridging the cells [74,126]. It has been widely demonstrated in the literature that high salinity of the marine environment effectively inhibits flocculation by a cationic polymer [74,127]. Moreover, the cationic flocculants polyacrylamide synthofloc 5080H showed the recovery of a marine microalga *Neochloris oleoabundans* greater than 90% with dosages of 30 mg/L. However, the effectivity of cationic polymeric flocculants decreases with increased salinity, due to a reduction in cationic charge [128]. The flocculants polyethylenoxide (PEO) sibfloc-718 together with a cationic flocculant polyacrylamide (PAA) showed sedimentation efficiency of about 80% in 120 min at a dosage of 0.025 and 0.015 g/l, respectively, without pH adjustment for harvesting the biomass of *C. vulgaris* GKV1 [123]. Moreover, the use of polyelectrolytes, such as PAA, can not be used to harvest microalgae because of its toxic effects [126]. Recently, enhanced flocculation efficiencies were achieved with recyclable polyamphoteric flocculants, because of their ability to adsorb to the assorted range of charge character in cellular suspensions as well as their flocculation efficacy over an extended range of pH values [129].

Besides several synthetic flocculants, some biodegradable, non-toxic organic flocculants derived or synthesized from natural sources such as chitosan is also effective in microalgal harvesting [130,131]. The microalga *Chlorella protothecoides* flocculation efficiencies > 95% were achieved with dense cultures (1 g/L algae dry weight) at cationic starch (degree of substitution 0.5) and chitosan dosages of 0.02 g/g algae dry weight [132]. Maximum flocculation efficiency of *Nannochloropsis salina* was achieved through chitosan precipitation and subsequent sweep floc at pH 8.0 [132]. The natural flocculants such as ecotan and tanfloc showed increased microalgae settling velocity, enabling over 90% biomass recovery in 10–20 min, with doses of 10 and 50 mg/L, respectively [131]. A novel water soluble copolymer flocculant AC-g-P (DMC-MACPPC), synthesized by grafting on to acryloyl chitosan backbone exhibited a high algal (*C. vulgaris*) flocculation efficiency of 73% at considerably very low concentration (20 ppm) of the copolymer [133]. The cellulose nanocrystals (CNC), derived from the acid hydrolysis of cellulose fibers, which is the most abundant natural and renewable organic polymer, can be used in microalgal flocculation [134,135]. Kan et al. [136] described a polymer-grafted CNC, which showed pH-responsive reversible flocculation and sedimentation properties. Recently, a new CNC that is a CO_2-switchable nanomaterial was demonstrated for the use in efficient microalgae recovery without any adverse effects on the downstream processing of biofuel production [137]. Moreover, further optimization of CNC dosage requirement is important to minimize the cost of the CNC for ecological sustainability and economic viability of this microalgal harvesting technology. Application of water soluble extracts of *Skeletonema marinoi* was found to promote flocculation of the microalga *Nannochloropsis oculata* [138]. A naturally occurring microbial flocculant poly (γ-glutamic acid) (γ-PGA) produced by *Bacillus subtilis* was used to harvest several oleaginous marine and freshwater microalgae with > 90% and > 95% flocculation efficiency, respectively, without damage to cell integrity [139]. Ndikubwimana et al. [140] also analyzed the harvesting of microalgae by applying the bacterial broth bioflocculant (γ-PGA) produced by *Bacillus licheniformis* CGMCC 2876. Recently, a freshwater microalga *Desmodesmus brasiliensis* was recovered with the flocculation efficiency above 99% after only 1 min of settling time using the bacterial broth bioflocculant (γ-PGA) produced by *B. licheniformis* CGMCC 2876 [101].

3.2.1.2.2. Inorganic flocculants. Microalgal flocculation with inorganic flocculants is based on charge neutralization, since microalgal cells are negatively charged due to ionization of surface functional groups [74]. There are certain disadvantages of using inorganic flocculants for microalgal separation, such as high pH sensitivity, generation of a large quantity of sludge due to profound use of inorganic flocculants for solid-liquid separation of the microalgae and casual contamination of end product due to use of metal salts [44,141]. Moreover, certain inorganic flocculants such as alum shows superior flocculating ability than those of ferric sulfate in terms of the optimal dose, pH sensitivity and quality of water slurry obtained. The increase in pH may influence the charge of microalgal cells and induces microalgal flocculation by promoting the precipitation of added flocculants [142,143]. Some freshwater microalgae such as *Chlorella vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. were harvested by increasing the pH value of the medium with flocculation efficiency of up to 90% [142]. The alkalizing agents such as phosphates, magnesium, sodium and calcium ions have been used to increase the pH levels required to begin chemical flocculation in microalgae [142–144]. Moreover, phosphate based thickening is mainly feasible for phosphate rich wastewater. The use of magnesium ions as a coagulant may be advantageous as it can be simply obtained from the wastewaters and shows similar coagulating effects to those of Al^{3+} and Fe^{3+} ions. Several microalgae can be effectively flocculated using the flocculants aluminum sulfate and ferric chloride; however, aluminum sulfate showed better flocculant efficiency over ferric chloride, requiring a lower dosage, owing to the relatively high surface charge density of the Al^{3+} ion compared to the Fe^{3+}, towards biomass concentration [145]. Limestone or dolomites can also be used, as per they carry magnesium ions, carbonates, hydroxides and oxides, offering the pH related coagulating activity [126,144]. Recently, the potential of some novel magnetic flocculants such as magnetic iron oxide (Fe_3O_4) nanoparticles have been shown for efficient harvesting of oleaginous microalgae [146,147].

3.2.1.2.3. Combined flocculation. Moreover, a combined multistep flocculation process using more than one type of organic and/or inorganic flocculants is also employed for algal harvesting. The marine microalgae were harvested by two flocculation processes such as combining polyelectrolytes with inorganic flocculants (e.g., ferric chloride or alum), and ozone oxidation followed by adding of flocculant [148]. Muylaert et al. [149] confirmed the feasibility of using cationic starch for flocculation of both fresh- and marine water microalgae. The combined use of ferric chloride ($FeCl_3{\cdot}6H_2O$) and polyacrylamide was shown to allow a significant increase in algal biomass (*C. vulgaris*) sedimentation rate up to 80% in the first five minutes. Furthermore, the flocculation efficiency of *C. vulgaris* achieved about 90% after 5 min of sedimentation, when adding of coagulant and flocculant mixture ($FeCl_3$ 50 mg/l + PEO based Sibfloc-718 7.5 mg/l) or flocculant with a ballast agent (Sibfloc-718 7.5 mg/l + 10% ballast agent i.e., flocculated biomass) [123]. The process of flocculation and centrifugation was assessed to harvest a culture of microalga *Scenedesmus* sp., and a concentration efficiency of 97.9% was achieved with 1.5 g/L of $Al_2(SO_4)_3$ at pH 8.5 and an average sedimentation velocity of about 2.7 cm min^{-1} [150].

Nevertheless, not a single harvesting process as mentioned above is cost-effective for biofuel production viewpoint, hence, applying a two-step process may prove the most effective ways to reduce the harvesting costs [151]. A low-cost primary harvesting process eliminates the majority of water from the algal broth; a slight load is left towards the secondary harvesting step. Subsequently, an energy-expensive but reliable and high throughput process of centrifugation can be applied as the secondary harvesting process without significantly affecting the overall production cost, as the major load is removed during the primary processing prior to centrifugation. The process of flocculation followed by gravity sedimentation may be a cheap approach for algae harvesting [144]. The energy demand would be greatly reduced if the microalgae could be pre-concentrated about 30–50 times prior to secondary harvesting by the use of centrifugation [15]. Overall, the harvesting costs of microalgae need to be reduced for their bulk production for biofuel. Apart from various technologies, application of membrane filtration also offers a promising approach towards microalgae harvesting in single as well as two-step processes [85] without the accumulation of chemicals from flocculating agents, as encountered in the flocculation technique. The combination of flocculation and sedimentation with centrifugation can considerably reduce harvesting costs [126,145]. Magnesium coagulation followed by gravity sedimentation or DAF has been used for harvesting both marine and freshwater microalgae [99]. Moreover, a dynamic membrane module in combination with coagulant dosing was applied to achieve one step microalgae harvesting for biodiesel production [152]. Several flocculants such as ferric chloride, chitosan, polyaluminum chloride was also experienced to improve the filtration process by controlling the membrane fouling and to concentrate the microalgal slurry in one step harvesting process [153]. Undoubtedly, microalgae have many reasonable advantages over other conservative biofuel sources [12], substantial developments are quiet required and necessary to implement several measures, especially towards efficient cultivation and harvesting technology for attaining the optimum algal biomass concentration intended for cost-effective biofuel production [12].

3.3. Biological methods (Bio-flocculation)

The process of bio-flocculation, using polymeric flocculants

produced by various microorganisms, is an emerging approach towards low-cost algae harvesting technique [115,154]. The effectiveness of bio-flocculation depends on the flocculation efficiency of used microbial flocculants as well as the behavior of the algal species [109]. The sticky polymer exopolysaccharides (EPS) play an important role in the microbial flocculation process. Both microalgae, and bacteria produce EPS are responsible for cell-cell adhesion to form the flocs. The efficiency of microbial flocculation depends on the extent of EPS synthesis by the used microorganisms and the competency of microalgae to attach to them to form flocs [141]. The involved mechanisms behind microbial flocculation may either be bridging or patching by the EPS excreted from flocculating microalgae [77,141,154]. It has been shown that under microbial flocculation, the accompanied microorganisms may also add in lipid yields [109,115] and subsequently, the resulting culture media can be reused, decreasing the costs of microalgal cultivation in the course of biofuel production [155]. Co-culturing of particular fungi was found to assist bioflocculation of some microalgae [155,156]. The fungal assisted flocculation process does not entail different cultivation conditions and allows reuse of culture medium without any supplementary treatment [155]. Some fungal species consist of over 30% lipid of total biomass can be appropriate for biodiesel feedstock in consort with the microalgal biomass [157]. Co-cultivation of fungi with *Chlorella vulgaris* by 1:2 ratios, respectively, was resulted into the removal of nearly 99% biomass after two days of co-cultivation [158]. Bioflocculation of precultured microalga *Chroococcus* sp. using a pellet forming filamentous fungus *Aspergillus lentulus* FJ172995, resulted in almost 100% harvesting within 6 h without addition of any nutrient or carbon source at the optimized fungal/algal (1:3) ratio [159]. Some bacterial species of different taxonomic groups are associated to the microalgal growth, also play a key role in microalgae flocculation [160]. Microbial flocculation by means of bacteria may require the addition of some chemical substrates [109] to enable their growth, but, this may cause unwanted microbial contamination to the microalgal culture medium. Therefore, harvesting of non-flocculanting microalgae adding flocculating ones can be an effective approach towards chemical free flocculation process [115]. A bio-flocculant produced by *Solibacilus silvestris* showed remarkable efficiency in flocculating microalgae without adding chemical coagulants [161]. Recently, an effective bioflocculation of microalgae *Chlorella sorokiniana* was achieved using the starved protozoan *Tetrahymena* sp. It was shown that under standard conditions of *Tetrahymena* to algae ratio (1:125) with 100 mM NaCl as an inducer of flocculation, allowed harvesting of > 95% of the biomass during 30 min of settling [162]. The process of bio-flocculation can be induced by nutrients (e.g., carbon and nitrogen) stressed condition for harvesting some species/strains of microalgae. Bio-flocculation can be combined with centrifugation or sedimentation to enhance the harvesting efficiency. Moreover, bio-flocculation is one of the cost-effective biomass harvesting technique which needs further research at pilot scale to carry out this process as an efficient harvesting technology [115,163]. Overall, if we keep our eye on different harvesting system, it seems that an efficient harvesting of algal biomass is still a technical bottleneck, and more pioneering research is needed to make the harvesting technology economically viable for microalgae-driven biofuel conversion.

4. Dewatering and biomass extraction

4.1. Dewatering/drying

Overall, an extensive literature survey has pointed out that the dewatering of microalgae is one of the main bottlenecks in algae cultivation. It has been estimated that around 84.9% of the total energy is consumed in the dewatering process [164]. Hence, optimization of a pre-concentration as well as dewatering and/or dehydration step is the most promising approach to improve the overall energy balance towards low-cost algal biomass and biofuel production [72,165,166]. The algal biomass accumulated after harvesting followed by dewatering through filtration and/or centrifugation as stated above further undergo de-hydration steps either by mechanical or thermal drying process to get the dry biomass for further downstream processes of lipid/oil extraction for the production of biofuels and co-products. The cost-effective drying process is expected to capitalize on the net energy production of the fuels [72]. The process of thermal drying where energy is primarily required as heat, consumes considerably more energy than mechanical drying/dehydration. Solar-drying may be a feasible route towards cost-effective drying process; however, this method seems impracticable due to the fact that this method is time consuming, requires comparatively large land area and depends on the environmental humidity and daily fluctuating sunlight. A new concept for mechanical dryer was proposed, alluring high drying efficiencies [167]. Moreover, the newly designed delta dryer techniques may have great potential towards energy efficient drying alternative; however, further development is needed to establish this concept as a realistic model [168]. Overall, recovery of a typical dry biomass concentration from algal paste is highly energy intensive and costly process. Hence, except for the extremely high-value products, wet algal biomass may be used in extraction of total lipids for biofuels.

4.2. Lipid extraction (oil from algae)

High-yield biomass with rapid growth rate in unit time, and putative high oil (triglyceride) content has favored microalgae towards an alternative source of fuel cells [169]. Several methods such as mechanical, physical, chemical or enzymatic are used for lipid extraction [56,170–172]. However, an efficient extraction technique is required for disruption of biomass prior to their further processing. It has been shown that the exerted temperature during lipid extraction affects not only the lipid content, but also the composition of specific lipids derived from the algal biomass [173]. Besides the dry route of oil extraction, another strategy of oil extraction in the water phase (wet route) has also been considered [172,174,175]. It has been specified that the dry route has a higher fossil energy ratio, while the wet route has extra potential in producing moderately high value end-product, biofuels [174]. Use of different organic solvents [176,177] and supercritical fluid [178,179] seems the most common and widely used methods for high yield algal lipid extraction. The solvent extraction of lipids using dry algal biomass is a usual process; however, due to energy intensive and costly system, drying is not feasible on a commercial scale for oil recovery aimed at various possible biofuels [180]. Consequently, use of wet biomass (~ 85% moisture) paste for solvent extraction of oils may be economically viable [176,177,181]. Recently, Chatsungnoen and Chisti [182] have analyzed the effectiveness of solvent mediated oil extraction from dry and wet biomass of six different microalgae from fresh- and marine water, and found that the oils could be extracted equally effectively from both freeze-dried and the paste samples. The Bligh and Dyer [183] method is one of the most common methods of solvent extraction of total lipids from algal biomass [184–186], where a mixture of different solvents of different polarities is used during the extraction process. In order to improve the basic Bligh and Dyer method, many modifications have been adopted [184]. A simple extraction process by cheap organic solvents without any pretreatment or co-treatment is seemingly the least expensive and most energy efficient options for oil recovery. Recently, a one-step extraction of the total lipids from the biomass paste of the microalga *Nannochloropsis salina* was optimized and more than 96% extraction of total lipids were found with the relatively reduced amount of mixed solvents (by ~ 48%) and extraction time (~ 78%) without any pretreatments or parallel treatments of the cell [177]. Recently, the use of some ionic liquids has been described to extract the lipids from microalgae [187–189]. Moreover, the use of different organic solvents or supercritical CO_2 has their own limitations. The organic solvents (e.g., hexane) used in lipid extraction is

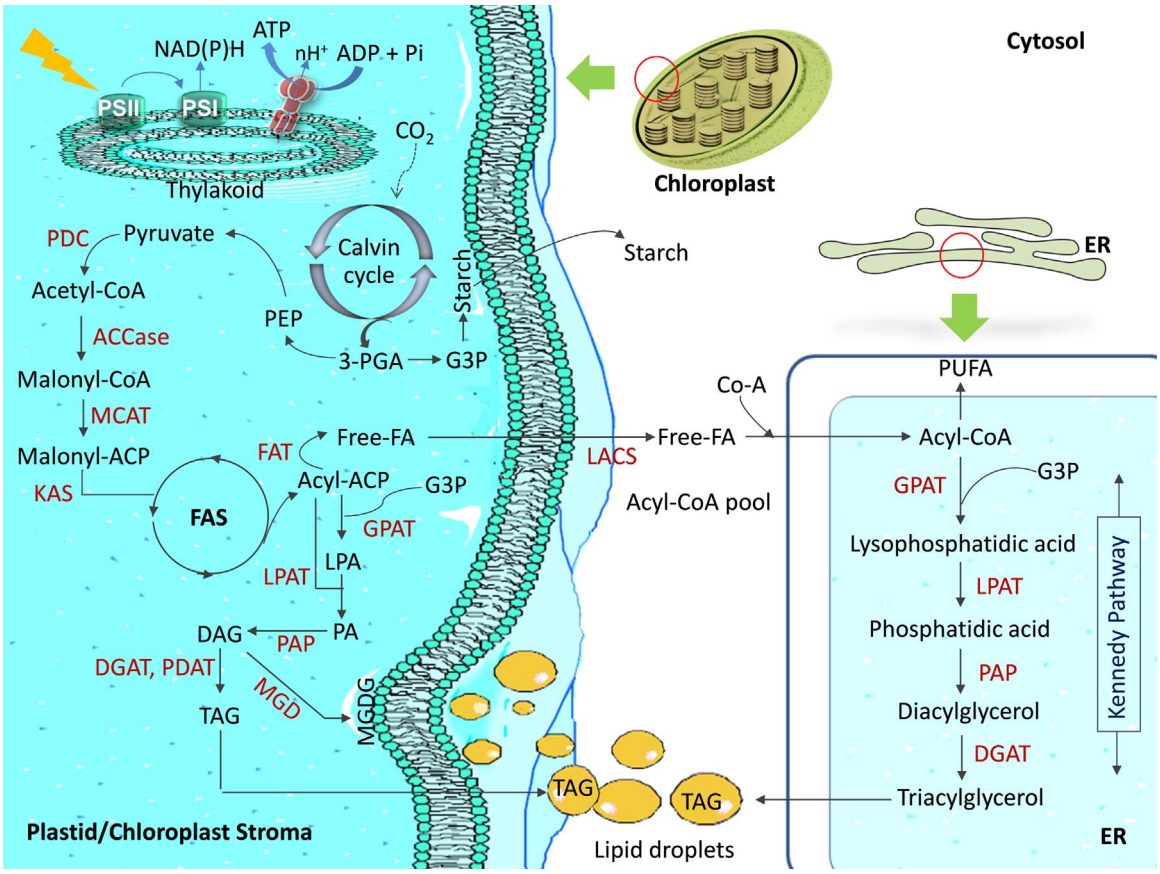


Fig. 5. A simplified view of lipid (TAG) biosynthesis in microalgae. Biosynthesis and accumulation of TAGs in prokaryotic microalgae take place via the chloroplast (thylakoids). [3-PGA, 3-Phosphoglycerate; G3P, glyceraldehyde 3-phosphate/glycerate 3-phosphate; PEP, Phosphoenol pyruvate; PDC, Pyruvate dehydrogenase complex; CoA, Cenzyme A; ACCase, Acetyl-CoA carboxylase; ACP, acylcarrier protein; MCAT, Malonyl-CoA:ACP transacylase; KAS, 3-Ketoacyl-ACP synthase; FAS, Fatty acid synthase (for fatty acid synthesis); FAT, Fatty acyl-ACP thioesterase, LACS, Long-chain acyl-CoA synthetase; PUFA, Polyunsaturated fatty acid; GPAT, Glycerol-3-phosphate acyltransferase; LPAT, Lyso-phosphatidic acid acyltransferase; PAP, phosphatidic acid phosphatase; DGAT, Diacylglycerol acyltransferase; TAG, Triacylglycerols; LPA, Lysophosphatidic acid; PA, Phosphatidic acid; DAG, Diacylglycerol; PDAT, phospholipid:diacylglycerol acyltransferase; MGD, monogalactosyldiacylglycerol synthase; MGDG, monogalactosyldiacylglycerol (For details see [218,259,269,274,276,288,301,319].

highly flammable and toxic, and their recovery is energy intensive [2]. The use of supercritical carbon dioxide ($ScCO_2$) needs high scale up cost in establishing and controlling the high pressure equipment [190]. Nonetheless, $ScCO_2$ is assumed as an important substitute for lipid extraction with organic solvents, as CO_2 is somewhat chemically inert, non-flammable, non-toxic, and produces a solvent free crude lipids without thermal degradation. High value lipid extraction by $ScCO_2$ has been conducted using both dry- [179,191] as well as wet-algae biomass [56,178,192]. Contrary to dry algal biomass, total lipid yield extracted from wet algal biomass was higher, suggested that by using $ScCO_2$ extraction, the energy used for the drying and milling step could be avoided [192]. Moreover, a recovery method by changing the nature of the solvent or process conditions could be an energy efficient alternative towards the efficient lipid extraction process. The CO_2 switchable solvent method [193] can be exceedingly promising for lipid extraction from dry/wet algal biomass [175], by implementing the low cost waste heat energy for the solvent recovery process. Overall, several methods are being used at a laboratory or commercial scale for lipid extraction [171], but none of the existing methods can be confirmed as a standard extraction methods. Further research is indispensable towards successful implementation of an economically viable extraction technology for achieving the maximum lipid yields for algal fuel.

The lipid extracted algal biomass residues can further be processed to produce a wide range of biofuels such as bio-ethanol, bio-butanol, bio-methane and biohydrogen [194,195]. These algal residues are rich in carbohydrates, proteins and pigments which could also be used as animal feed, and co-production of some high value products.

5. Biofuels from algae

Algae, including cyanobacteria are potentially sustainable source of liquid as well as gaseous biofuels, including biodiesel, bioethanol, biohydrogen, and biomethane. It is conceivable to produce suitable algal biofuels at commercial scale to fulfill the global energy demand [196]. Several species/strains of microalgae have been employed as an ideal source of biofuels owing to their excellent photosynthetic efficiency to fix the light energy into chemical energy, high CO_2 sinking capacity, fast growth cycle under limited nutrient requirement and, able to survive under a wide range of climatic conditions and high lipid content with year round production. It has been estimated that oil productivity of microalgae exceeded up to 12,000 L ha^{-1} for biodiesel yield at 30% triacylglycerides (TAG) than the biodiesel yield 1190 L ha^{-1} of the best oilseed crops rapeseed [78]. Many species of microalgae have high lipid content ranging from 20% to 80% of their dry weight [14,197,198]. Microalgae are reported to produce about 15–300 times more oil for biodiesel production than those of traditional crops on an area basis [14,78] (Fig. 2). The residual biomass after oil extraction for biodiesel production, can be used to derive several valuable co-products, or fermented to yield ethanol or methane gas. Some microalgae are capable of photobiological production of biohydrogen gas. Moreover, several lines of study suggest that algae could be the most competent natural resource for liquid or gaseous biofuel production. In this review, the algae-based biodiesel production has been targeted and briefly discussed.

5.1. Biodiesel from algae

Biodiesel is one of the most important energy fuels in the global market. As a result of its high demand, and dependency on vegetable oils for biodiesel production has aroused serious concern about the availability of feedstocks to the biodiesel industry. Moreover, lack of vegetable crop oil as feedstocks for biodiesel production has pressurized the world's biodiesel industry to opt an alternative feedstock for oil production, derived from non-food sources [199]. Fortunately, a deep understanding of the microbial chemical diversity and the search for alternatives has resulted into an advent of intriguing new option- microalgae as an efficient source of biofuels. Using the algae oil as a source of biodiesel can overcome the limitations of first generation biofuels for energy needs with no impact on the food chain. Several oleaginous microalgae are considered the most efficient and important renewable fuel crops due to high quantity of lipids mainly triacylglycerides (TAGs). TAGs are one of the most suitable lipids that can be used as a potential feedstock for biodiesel conversion [7,195]. The neutral lipids, commonly in the form of TAG, accumulate in the form of lipid vesicles and so-called oil bodies in the cell cytoplasm (Fig. 5). The fatty acid composition of these photosynthetic organisms is comparable to vegetable oils and thus is being used as a competent substitute for plant oils to make an effective and clean fuel for diesel engines [200]. Conversion of algal oil into biodiesel as end-product is fairly cost effective approach than the methods used for conversion to other fuels.

Biodiesel from algae is supposed to be a potential substitute for petroleum-derived diesel fuels due to its overall performance and fuel quality [201]. It is a renewable transportation fuel consisting of methyl and ethyl esters of fatty acids. The major constituents of biodiesel are straight fatty acid chain such as palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acid (Table 3). Various studies have revealed that fatty acid composition greatly affects the fuel properties and quality of biodiesel. Moreover, there are significant differences in combustion and transportation properties of biodiesel and petroleum-derived diesel fuels due to significant differences in their physicochemical properties [202,203]. In contrast to conventional diesel, biodiesel includes higher oxygen content and lack of aromatic hydrocarbons and sulfur leading to its proper combustion, reducing the carbon monoxide (CO) production and emission of particulate matter (PM) in the atmosphere. However, there is contradictory report regarding the use of biodiesel and increased emission of PM in the atmosphere [204,205]. Furthermore, biodiesel comprises a higher flash point and better lubricity, and can be blended with petroleum diesel fuel to improve the lubricity of diesel and increases the cetane value [206]. In addition to ignition/combustion characteristics as expressed by the cetane number, some other properties such as viscosity, density, oxidative stability, heat of combustion, cold filter plugging point, solidifying point are taken into consideration in determining the suitability of biodiesel as a clean energy [207]. Biodiesel from microalgal oil were found comparable to those of diesel fuel with the limits established by the American society for testing and materials (ASTM) [208]. In fact, the biodiesel from microalgae showed much lower cold filter plugging point of − 11 °C in comparison with the petrodiesel fuel [209]. Higher viscosity and cloud point is one of the most important and sensitive issues for transportation fuel. Contrary to petroleum-derived diesel fuels, higher viscosity of biodiesel fuel may cause larger droplet size during injection and can affect fuel injection parameters. Nonetheless, combined bioengineering and transesterification could be a viable and effective method for the high quality biodiesel production from microalgal oil. Moreover, microalgae biodiesel satisfies European Biodiesel Standards (EN 14214) except its low cetane number that can be compensated by mixing microalgae biodiesel with petrodiesel fuel [201]. Overall, biodiesel produced from algal oil is virtually analogous to the usual biodiesel in terms of the key physico-chemical properties [200,208,209] that have a relatively less adverse impact on air quality and human health [210].

5.1.1. Induction of the synthesis/accumulation of TAGs

It is more important to select the suitable algal species/strains having great potential to accumulate considerable lipid contents for high biodiesel production [211,212]. Besides, inherent capacity of algae to produce lipids, a number of abiotic factors such as nutrient starvation (e.g., -N, -P, -S, and -Fe), light, temperature, pH, salinity and dissolved oxygen may affect TAG accumulation in the algal cells [14].

It has been shown that under normal growth conditions, the lipid content in algae varies from 5% to 30% of dry weight; however, under nutrient stressed conditions of nitrogen deficiency, the lipid content mainly TAGs may reach up to double or triple [7,14,78]. Several microalgae can synthesize 20–50% TAGs of their dry cell weight, under different environmental conditions [7,14]. Nitrogen (N) starvation is usually considered most effective for increased TAG accumulation in microalgal cell [214,219,220]. The decline in nitrogen concentration by 75% in the growth medium has ensued about 100% increase in the total lipid content in the microalga *Nannochloropsis oculata* [213]. Moreover, the microalga *Nannochloropsis* may be one of the best feedstocks for biodiesel production because of high lipid content ranging from 37% to 60% of dry biomass, higher than other microalgal strains [217,221,222].

In response to nitrogen starvation, upregulation of certain genes liable for neutral lipid accumulation was also observed in diatom [223]. It has been suggested that the capacity to accumulate high TAG levels in green algae critically depends on their ability to divert carbon flow towards acetyl-CoA. Increased level of acetyl-CoA followed by enhanced TAG biosynthesis was observed in the green algae *Chlorella desiccat, Chlamydomonas reinhardtii* and *Dunaliella tertiolecta* under N-deprived condition [224]. The key enzymes of acetyl-CoA carboxylase, diacylglycerol acyltransferase and NADPH involved in lipid biosynthesis were up-regulated about 3–13 times by high light and/or nitrogen deficiency, inducing the TAG biosynthesis in a marine alga *Nannochloropsis oculata* [225]. Exogenous supply of carbon through acetate boost [226], free-FA supplementation or use of starch-less mutants [227,228], strongly enhances TAG accumulation in microalgae [229]. The starch-less mutant of *Chlamydomonas reinhardtii* (BAF-J5) was found to accumulate lipids up to 65% of dry cell weight when grown photoheterotrophically and subjected to nitrogen starvation [230]. The Light regimes also play an important role as it has an obvious effect on the lipid production and fatty acid composition in algae [216,231]. In general, low light intensity favors the formation of chloroplast associated membrane lipids, while high light intensity promotes the accumulation of neutral storage lipids specifically TAGs in algal cells. The storage lipid TAGs are considered more superior biodiesel feedstocks than phospholipids or glycolipids as a consequence of their higher percentage of fatty acids and lack of phosphate [7]. Increased accumulation of lipids has been observed in algae at their stationary growth phase than at logarithmic phase [232,233]. Besides nutrient and light regimes, salinity [215,234], pH and temperature [213,235] also affect the lipid metabolism in algae. Moreover, the effect of different environmental factors on growth, lipid content and fatty acid composition vary differently across different taxonomic groups of algal species/strains (Table 3), and more efforts are still needed to clarify the key cellular mechanisms towards enhanced lipid production by key physiological factors.

5.1.2. Conversion of oil/TAG to biodiesel

Biodiesel is typically produced by transesterification process, where TAG/Free FA react with a mono-alcohol (e.g., methanol or ethanol) in the presence of a suitable catalyst to form low molecular weight fatty acid alkyl esters [70,236–239]. Generally, methanol is preferred for transesterification, as it is inexpensive and more reactive than ethanol [238]. The transesterification of triglycerides can be catalyzed either by acids (e.g., Hcl, H_2SO_4, H_3PO_4 and sulfonic acid), alkaline (e.g., KOH, NaOH and CH_3ONa) or enzymes (e.g., lipase); however, alkali-catalyzed transesterification is commonly used for industrial production of

Table 3
Some important fatty acid content of triacylglycerols or oil/neutral lipids from different microalgal species.

Algae	Growth condition	Fatty acids (%)										References
		16:0	16:1	16:2	16:3	16:4	18:0	18:1	18:2	18:3	18:4	
Chlamydomonas reinhardtii	Medium containing 0 mM NH_4^+	41.8	3.1	0.9	5.1	–	1.5	27.3	10.0	8.8	1.4	[296]
Haematococcus pluvialis	Grown under high irradiance	23.9	–	0.5	1.6	2.4	1.5	31.6	24.5	7.0	1.4	[299]
Eremosphaera viridis	Grown under N-deficient conditions.	8.0	–	–	0.7	–	1.5	67.0	16.6	–	1.1	[337]
Dunaliella salina	Grown under N-deficient media	25.6	1.6	0.6	1.2	6.2	2.2	24.1	16.3	20.4	–	[300]
Chlorococcum sp. R-AP13	Grown in fresh MA medium	39.7	3.6	–	–	–	8.1	22.0	3.2	7.5	–	[113]
Nannochloropsis salina	Cultivated in *f*/2 medium	~40	38–40	–	–	–	1.4–1.6	> 6	0.9	–	–	[328]
Microcystis aeruginosa	–	47.6	2.3	–	–	–	3.9	22.4	2.4	2.4	–	[338]
Spirulina maxima	–	32.1	–	–	–	–	10.9	2.6	9.9	12.8	–	[338]
Parietochloris incisa	Grown on BG11 medium under an air/CO_2 (99:1) atmosphere	13.3	0.5	< 0.1	0.4	–	3.7	15.3	10.4	2.1	–	[232]
Nannochloropsis sp. F & M-M24	N-deprived medium	44.49	30.54	–	–	–	1.37	12.83	1.10	0.23	–	[339]
Tetraselmis suecica F & M-M33	nitrogen and phosphorus starved condition	31.34	2.85	–	0.92	4.30	1.42	43.06	4.78	6.51	0.94	[339]
Chlorella vulgaris	N-deficient conditions	17	1	3	6	–	2	47	10	14	–	[340]
Chlorella zofingiensis	N-deficient conditions	15	1	4	2	–	3	47	17	8	–	[340]
Neochloris oleoabundans	N-deficient conditions	23	3	3	2	–	3	41	21	3	–	[340]
Scenedesmus obliquus	N-deficient conditions	14	5	4	3	1	5	50	7	8	1	[340]
Nitzschia laevis	Grown in a modified Lewin's marine diatom medium	34.1	25.4	–	–	–	0.1	5	3.1	2.9	–	[341]
Chlorella vulgaris	Bold's Basal Medium under nutrient replete condition	23.1	0.2	7.4	5.8	–	5.2	16.1	20.9	18.0	–	[342]
Scenedesmus sp.	Bold's Basal Medium under nutrient replete condition	24.5	2.1	2.3	6.0	–	4.9	19.8	34.2	2.5	–	[342]
Cylindrotheca fusiformis	Walne's medium under nutrient replete condition	42.8	29.0	–	0.3	–	0.5	16.4	1.2	–	–	[342]
Nannochloropsis sp.	*f*/2 medium under nutrient replete condition	40.9	26.3	–	–	–	1.0	7.0	1.5	–	–	[342]
Tetraselmis subcordiformis SHOU-S05	N supplemented f/2 medium	12.55–17.45	0.62–0.89	1.90–2.75	12.68–18.59	–	0.08–0.14	7.12–11.44	10.70–16.22	0.61–22.22	3.17–4.77	[343]
Nannochloropsis oculata SHOU-S14	N supplemented f/2 medium	13.99–16.64	17.92–20.00	1.91–2.98	0.46–0.54	–	0.26–0.84	3.04–8.26	3.89–4.26	–	–	[343]
Pavlova viridis SHOU-S16	N supplemented f/2 medium	14.34–26.92	19.82–26.56	0.73–1.99	0.58–0.87	–	0.13–0.52	1.85–5.66	0.73–1.85	1.00–2.37	2.93–4.03	[343]
Nannochloropsis sp.	f/2 medium	27.3–38.6	21.4–24.1	–	–	–	0.8–1.0	5.1–10.7	1.6–2.1	–	–	[344]
Chlorella vulgaris	CHU-13 medium	34.91–42.54	0.32–1.42	–	–	–	0.91–2.70	19.20–29.92	11.87–18.29	6.53–12.92	–	[185]
Chlamydomonas reinhardtii BAF-J5	TAP (N-) medium	19.389	1.645	0.733	1.583	2.093	1.999	15.802	10.545	10.254	–	[230]
Chlorella protothecoides	Polytoma medium under different light intensity	11.80–14.86	2.55–3.31	–	–	–	2.55–3.86	11.14–32.19	31.51–35.26	7.44–12.34	–	[345]
Scenedesmus abundans	Liquid culture medium under defined (4000–6000 lx) light intensity	48.34–62.62	3.38–5.16	–	–	–	1.51–4.63	20.0–29.09	0.65–1.80	1.0–5.67	–	[346]
Choricystis minor	WC (Wright's cryptophyte) medium	23.18	1.8	0.68	2.21	–	2.69	44.86	6.81	8.14	2.50	[347]
Scenedesmus obliquus	BG-11 medium containing different nitrate concentrations (0–1.5 g/L)	22.32–26.39	3.30–5.88	1.25–1.78	–	–	1.56–3.63	57.29–60.59	5.63–9.34	–	–	[348]
Chlorella pyrenoidosa	BG-11 medium containing different nitrate concentrations (0–1.5 g/L)	26.19–34.18	5.24–11.42	0.61–3.47	–	–	1.53–4.62	0.0–35.87	18.76–24.43	32.43–36.34	–	[348]
Coccolithophora sp.	ASM medium with different salinity (7.5–60%).	13.7–26.6	2.0–9.6	–	–	–	2.6–19.1	3.7–8.6	2.2–7.0	2.7–6.4	10.3–25.7	[349]
Prymnesium parvum	ASM medium with different salinity (7.5–60%).	7.1–17.0	5.0–10.1	–	–	–	3.3–4.6	6.6–10.0	1.7–5.2	2.6–7.1	20.7–30.9	[349]

biodiesel due to relatively much higher reaction rate [237,240]. Different catalysis techniques used in transesterification reaction have recently been reviewed [70,241–243]. In-situ transesterification is considered as an unconventional route of biodiesel production, as this technique eliminates the need for lipid or oil extraction prior to transesterification [243]. The alcohol combines with the triglycerides to form fatty acid esters and glycerol [244]. The glycerol produces as a byproduct in biodiesel industry can be converted into hydrogen gas by anaerobic fermentation [245]. Moreover, TAGs are comprised of three long chain fatty acids esterified to a glycerol backbone, once react with an alcohol, the three fatty acid chains are released from the glycerol skeleton and combine with the alcohol to form fatty acid alkyl (methyl or ethyl) esters (biodiesel).

In general, water content of the algae oil is removed at high temperature and allowed to cool. Meanwhile, an alkali catalyst (e.g., NaOH or KOH) is mixed with and a mono-alcohol (e.g., methanol) in a catalyst tank and stirred to produce sodium methoxide (CH_3NaO) by the deprotonation of methanol. Subsequently, clean oil is further moderately heated and mixed with the CH_3NaO and transferred to ultrasonic or mixer equipment. After adequate mixing, the solution is allowed to cool to settle the biodiesel. Being immiscible, fatty methyl or ethyl ester (biodiesel) is easily separated from glycerol and comes out on the top surface, and glycerin as a byproduct settled in the bottom part [246]. Finally, the formed biodiesel is separated and washed properly to remove the impurities such as unfiltered particulates, methanol, and glycerin by different methods.

The process of conventional transesterification for the conversion of fats/oil to biodiesel may often cause soap formation, and consumes catalyst leading to decrease of catalyst efficacy and biodiesel conversion rate [247,248]. Hence, a catalyst-free, supercritical methanol method could be readily used for biodiesel fuel production in a simple manner. This method proved to produce a high yield, due to concurrent reactions of transesterification of triglycerides and methyl esterification of free fatty acids [247–249]. The formed ester fuels/biodiesel produces slightly lower power and torque than diesel; however, it is non-toxic, biodegradable and has low emission profiles that can replace petroleum-based diesel fuel [250].

6. Microalgal lipid biosynthesis

It is very important to understand the intracellular reaction networks and machinery involved in a metabolic progression during the normal growth of an organism. The various life-sustaining metabolic processes such as lipid metabolism can be fundamentally different amongst various organisms including microalgae or cyanobacteria. Moreover, the basic pathway of TAG biosynthesis is assumed almost analogous to those established in higher plants. Unlike higher plants, the complete pathway of CO_2 fixation for TAG synthesis and their sequestration takes place within a single algal cell [7,251].

In general, lipid biosynthesis is an energy intensive process, and subject to the generation of CO_2 mediated several intermediate carbon compounds over the Calvin cycle using ATP as energy, and reducing power (e.g., NADPH) formed during the light reaction of photosynthesis. Several enzymes have been reported responsible for TAG synthesis [252]. The most important pathway for the production of neutral or storage TAG, and structural lipids or membrane glycerolipid in algae involves *de-novo* synthesis of fatty acids in the chloroplasts. Briefly, a three-carbon compound glyceraldehyde 3-phosphate (G3P) is converted into acetyl-CoA (Ac-CoA) through glycolysis process. It was proposed that the increased accumulation of TAG critically depends on the enhanced production of Ac-CoA in the oleaginous green alga *Chlorella desiccate* [224]. The formed acetyl-CoA is further carboxylated to form malonyl-CoA, the central carbon donor for fatty acid synthesis, in the presence of acetyl-CoA carboxylase (ACCase) [253]. Two different forms of ACCase (i.e., homomeric and heteromic), found either in the plastid or in the cytosol, is assumed to control the rate of *de-novo* fatty acid synthesis and elongation pathways in both plant and algae [254–258]. However, it has been assumed that ACCase does not catalyze a rate-limiting step in algae [259,260]. The malonyl moiety is transacylated to ACP (to form malonyl-ACP) by malonyl Co-A-acyl carrier protein (ACP) transacylase (MCAT) [261]. Further elongation of the fatty acids via extension of the acyl chain with malonyl-ACP is catalyzed by the enzyme fatty acid synthase (FAS) [262]. The initial step of FAS reaction is catalyzed by the condensing enzyme 3-ketoacyl-ACP synthase (KAS) to form 3-ketoacyl-ACP (3-KA-ACP) [263]. The FAS catalyzed elongation of fatty acids is accomplished by three subsequent steps, i.e., synthesis of 3-hydroxyacyl-ACP (3-HA-ACP), transenoyl-ACP (t-E-ACP) and the final product acyl-ACP, with enzymes 3-ketoacyl-ACP-reductase (KAR), 3-hydroxyacyl-ACP-dehydratase (HD) andenoyl-ACP-reductase (ENR), respectively [264]. The fatty acyl-ACP formed in the chloroplast act as precursors for the synthesis of both structural or neutral (i.e., TAG) lipids. Furthermore, the elongation of fatty acids is primarily completed by two ways in the chloroplast: a) the removal of acyl group from ACP, synthesizing different fatty acid with glycerol 3-phosphate (G3P) by the enzyme glycerol-3-phosphate acyltransferase (GPAT); b) the fatty acyl-ACP thioesterase (FAT), located in the inner membrane of chloroplast, hydrolyzes the thioester bonds of acyl-ACP to release free fatty acids (FFAs) [264]. Fan et al. [265] established that TAG synthesis is dependent on the *de-novo* synthesis of fatty acids in the chloroplast of N-starved *Chlamydomonas* sp. The enzymes ACP, KAS and FAT have been shown to play an important role in fatty acid synthesis in the microalga *Haematococcus pluvialis* [255]. The formed FFAs is subsequently transported to the outer membrane of the chloroplast in the cytosol [266,267] and re-esterified with chloroplast outer membrane enzyme long-chain fatty acyl-CoA synthetases (LACS) to produce acyl-CoA pool in the cytosol [268,269]. The acyl-CoA enters the endoplasmic reticulum (ER) to be converted into polyunsaturated fatty acids (PUFAs), and also serves as a substrate for intermediate products of TAG biosynthesis through the sequential transfer of acyl groups from acyl-CoA [256,270,271].

In both chloroplast and ER, biosynthesis of TAG takes place by means of Kennedy pathway, where GPAT may serve as a key enzyme in algae that catalyzes the allocation of a fatty acid from acyl-ACP or acyl-CoA to G3P, producing the LPA [265,272–274]. Moreover, Kennedy pathway involves a series of enzymatic steps in the production of some intermediate products such as lyso-phosphatidic acid (LPA), phosphatidic acid (PA) and diacylglycerol (DAG), with glycerol-3-phosphate acyltransferase (GPAT), lyso-phosphatidic acid acyltransferase (LPAT) and phosphatidic acid phosphatase (PAP), respectively [269,273,275,276]. The enzyme LPAT catalyzes the esterification of LPA to form PA [252,276]. However, LPAT has only been reported in some green microalga such as *Micromonas* sp. [267] and *Scenedesmus dimorphus* [277]. Recently, Yamaoka et al. [278] have reported a plastid-targeted 2-lysophosphatidic acid acyltransferase (CrLPAAT1) in *Chlamydomonas reinhardtii* that acylates the sn-2 position of an LPA to form PA, the first common precursor of membrane and storage lipids. The PA is further dephosphorylated by PAP, forming DAG, a precursor for the synthesis of TAG and membrane lipids [252,265,279,280]. The genes encoding plastidic PAP have been proposed to play a crucial role for TAG synthesis in the green alga *C. reinhardtii* [273,279]. Finally, DAG is acylated into TAG, using diacylglycerol acyltransferase (DGAT) [281]. Several isoforms of DGAT have been reported in algae [254,275,279,282–287]. The PA and DAG formed as an intermediate of the Kennedy pathway may also assist the synthesis of membrane lipids or phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and glycolipids, etc. [267,274,288].

An acyl-CoA independent pathway for the synthesis of TAG has also been proposed in algae, which utilizes phospholipids and DAG as an acyl donor and acceptor, respectively, using an enzyme phospholipid-diacylglycerol acyltransferase (PDAT) [7,282,289]. It was proposed that PDAT protein (CrPDAT) plays an important role in the turnover or

recycling of membrane lipids such as monogalactosyldiacylglycerol (MGDG), sulphoquinovosyl diacylglycerol (SQDG) and PG into TAG under N-starvation conditions in *Chlamydomonas* sp. [227,265,282,289]. Furthermore, a plastid galactoglycerolipid degradation 1 (PGD1) was found to involve in TAG accumulation by hydrolyzing the membrane lipid MGDG with an increased pool of fatty acids in *Chlamydomonas reinhardtii* growing under N-starvation [290].

The primary storage compound starch formed in algae is also associated with the accumulation of neutral lipids in the cell through the citric acid cycle occurring in the mitochondrion [269,288,291–295]. Overall, TAG synthesized through various pathways is largely accumulated in the cytosol of algal cell in the form of lipid droplets/bodies (LDs/LBs) (Fig. 5). The size of LDs varies in different species of algae, depending on their growth stage and environmental conditions [296,297].

Furthermore, synthesis and accumulation of the lipid body may also occur in the inter-thylakoid space of the chloroplast in some green algae. In microalgae, the lipid droplets contain about > 90% TAG of total lipids [298], with palmitic (C16:0), oleic (C18:1), and linoleic acids (C18:2) as the major fatty acids (Table 3) [296,299,300], that can be employed in biodiesel production.

Moreover, an intensive research has recently been focused on the biosynthesis and accumulation of TAG in algae [40,218,259,269,274,288,301]. However, a precise understanding of lipid biochemistry, and how the cellular machinery work during lipid metabolism, in particular biosynthesis or the accumulation of fatty acids and TAG, are rather scarce for microalgae. A basic and simplified scheme for TAG biosynthesis in algae is shown in Fig. 5.

7. Genetic manipulation of algae for enhanced lipid/TAG production

A number of engineering processes have been adopted for enhanced production of algal biofuels, including biodiesel [302]. To date, most of the effort in genetic engineering has been focused on developing new genetically modified algal strains for enhanced photosynthetic efficiency and increased carbohydrates and/or lipid contents in different algal species, through the cellular expression or down regulation of various genes encoding a specific enzyme [11,259,274,277,286,303–305]. Various studies have proposed a connection between the rate of fatty acid (FA) and TAG biosynthesis to the accessibility of specific carbon precursors. In most oil-accumulating oleaginous algal species, major metabolic control for TAG biosynthesis takes place at the level of FA biosynthesis that may be limited by the rate of carbon flux towards plastids/chloroplast [225,294]. It was found that the knockdown of a citrate synthase gene, which incorporates acetyl-CoA into the citric acid cycle, led to a major increase in TAG accumulation in the green alga *Chlamydomonas reinhardtii* [294]. Moreover, it has been suggested that over expression of the rate-limiting enzymes in Co-A biosynthesis in chloroplasts may have significant implications for future genetic manipulation to enhance FA and TAG biosynthesis in microalgae [224].

An integrated genomic and transcriptomic studies have revealed that TAG synthesis in algae occurs through a set of metabolic steps with the investment of various specific genes and their products that are functional homologous to plant genes [306]. Several studies have been carried out using genetic and proteomics approaches to identify the key genes involved in TAG accumulation, using some model microalgae such as *Chlamydomonas* and *Nannochloropsis* sp. [273,286,305]. An increase in TAG accumulation was observed in *C. reinhardtii* following nitrogen deprivation with an increase in transcript abundance of FAS complex genes, including those encoding ACP and Fatty acyl-ACP thioesterase 1 (FAT1), signifying that ACP and FAT may be directed to improved lipid production in microalgae [279]. Contrary to FA synthetic genes, manipulation of the Kennedy pathway genes can be a promising strategy for enhanced TAG production. Recently, GPAT-like gene (*LiGPAT*) from the oleaginous green microalga *Lobosphaera incise* was overexpressed in *Chlamydomonas reinhardtii* which resulted in a 50% increase of TAG content on a cell dry weight basis as compared to the control culture without negative impact on growth parameters [307]. Phospholipid-diacylglycerol acyltransferase (PDAT) and the type-1 and type-2 diacylglycerol acyltransferases (DGATs), were up-regulated under N-starvation in *Chlamydomonas reinhardtii*, and up to ~ 25% of the total TAG accumulation was observed due to the turnover of chloroplast membrane lipids, predominantly monogalactosyldiacylglycerol (MGDG), sulfoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG) [282,289]. A number of microalgal acyltransferases such as DGAT and GPAT have been explored in metabolic engineering approaches to increase TAG production via heterologous or homologous expression [307–309]. Moreover, the biochemical functions of *DGTT1* and *PDAT1* were confirmed by restoration of TAG accumulation in a yeast strain lacking all acyltransferase activity [282]. The heterologous expression of yeast-derived TAG biosynthesis-related genes such as GPAT, LPAT, PAP, DGAT, and G3PDH, resulted in a two-fold increase of TAG accumulation in *Chlorella minutissima* [310]. Expression of LPAAT (CrLPAAT1) in plastids led to a > 20% increase in oil content in *Chlamydomonas reinhardtii* under nitrogen-deficient conditions [278]. Overexpression of a type-2 DGAT was found to increase TAG accumulation by 35% in the marine diatom *Phaeodactylum tricornutum* [308]. Deng et al. [283] have studied five different DGAT-2 homologous (CrDGAT2) genes on lipid accumulation, and found that RNAi silencing of *CrDGAT2*-1 or *CrDGAT2*-5 ensued a decrease in lipid content, while transformants harboring CrDGAT2-4 showed increased lipid content in the green alga *Chlamydomonas reinhardtii*. However, La Russa et al. [284] did not found an increase in lipid accumulation upon overexpression of three type-2 DGAT (*DGTT1* to *DGTT3*) homologue genes *CrDGAT2a*, *CrDGAT2b*, and *CrDGAT2c* in *Chlamydomonas reinhardtii*, growing under normal or nitrogen/sulfur deprived conditions. Moreover, Iwai et al. [309] observed the enhancement of TAG in a wild type *Chlamydomonas reinhardtii* by overexpressing a type-2 DGAT (*DGTT4*) with a phosphorus starvation-inducible promoter sulphoquinovosyldiacylglycerol 2 (SQD2). The overexpression of DGAT (*BnDGAT2*) from *Brassica napus* resulted enhanced neutral lipid biosynthesis in *Chlamydomonas reinhardtii* [311]. Increased production of FA/TAG was found in some starchless mutants such as *Chlorella pyrenoidosa* STL-PI [312], *Chlamydomonas spp.* [291,292,298] and *Scenedesmus* obliquus [295,313]. Recently, genes involved in FA and TAG biosynthetic pathway were investigated in three different strains of *Scenedesmus* sp. through molecular cloning, transcriptome mining and expression profiling. Six different genes such as *ACP*, *FATA*, *KASII*, *LPAAT*, *PAP* and *DGAT* was considered potential targets to design novel strain by metabolic engineering for enhanced lipid production [277].

Furthermore, downregulation or targeted gene knockdown of certain enzymes such as lipase, phospholipase or acyltransferase has resulted in an increase in lipid accumulation in diatom [223,314]. The genome of some species of *Nannochloropsis* was explored to assist research on gene functions, targeting the metabolic engineering pathways for substantial amounts of TAG synthesis [285,286,305,315]. Transcriptomic studies of a *Nannochloropsis* sp. growing under nitrogen deprived conditions exhibited up-regulation of genes encoding putative DGATs, and some other genes involved in *de-novo* fatty acid biosynthesis intended for improved TAG accumulation [305]. Moreover, smart understanding of various genes and/or enzymes at a biochemical and molecular level will be a vital step towards the genetic engineering of the fatty acids/TAG biosynthesis in algae.

No doubt, numerous attempts have been made towards the metabolic engineering intended for increased TAG accumulation in different algae [274,280,288,307,316,317]. Identification of regulatory network hubs that control lipid metabolism [280,318] as well as the recent development of molecular techniques to interrogate and edit the nuclear genome may lead to engineer superior oil-accumulating algal strains glycerolipid synthesis [288,317]. Recently, Goncalves et al. [319] have uncovered the role of a transcription factor ROC40 in N-starvation induced accumulation of TAG in the green alga *Chlorella* sp.

UTEX29. Upregulation and overexpression of an *Arabidopsis* NAD(H) kinase resulted in a substantial increase (~ 110.4%) in lipid production in the oleaginous green microalga *Chlorella pyrenoidosa* [320].

Moreover, genetic manipulations of microalgae for enriched lipid (TAG) production are still under early stages, since a number of genetic engineering approaches were found ineffective towards improved fatty acid biosynthesis in microalgae [321,322]. Upregulation of the enzyme ACCase did not improve fatty acid synthesis in some diatoms such as *Cyclotella cryptica* and *Navicula saprophila*, indicating that mere a single gene or product is not responsible for the synthesis of fatty acids [259,293,323]. Furthermore, no correlation was observed between ACCase activity and TAG accumulation in the microalgae *Chlorella vulgaris* and *Chlorella sorokiniana* cultivated under different growth conditions [260,324]. Genetic and metabolic engineering may have an impact on cell growth, since it has been observed that algal mutants with improved TAG synthesis confronted slower growth rates [291] that may cause increased risk of culture contamination. The manipulation of FAS system was found to affect the fatty acid composition, but not their accumulation in microalgae [261,325]. Overall, considering the above facts, more extensive study is yet needed to explore the exact role of different cellular machinery involved in the lipid biosynthestic pathway for successful genetic engineering approach towards increased triacylglycerol production in different taxonomic groups of algal species.

8. Conclusion and future perspectives

Biofuels produced from algae may be one of the reliable alternatives over limited resource of fossil fuels. Biodiesel production from algae may offer an ecologically as well as economically sustainable solution towards globally increasing energy requirements. Biodiesel is a non-toxic and biodegradable fuel that can be produced from many different feedstocks, including algal oil/fatty acids. Different technologies are being used in the industrial production of biodiesel fuel using fatty acid/TAG. Recently, an integrated system has been developed for mass cultivation of microalgae using the wastewater and flue gas, aimed at biological purification of municipal wastewater and greenhouse gas (CO_2) mitigation. Moreover, biodiesel is a clean and alternative energy that has been suggested as the energy carrier of the future. Biodiesel production from algae has attracted public interest due to its potential as a renewable energy carrier. However, at present, the level of biodiesel production is not sufficient and economically not viable as a competitive energy carrier.

Current research efforts are mainly focused on changes in culturing conditions and strain improvement by systems metabolic engineering to enhance the synthesis of fatty acids/TAG for biodiesel production. Successful applications of systems metabolic engineering based on genomic, transcriptomic, proteomics and metabolomics may allow the development of suitable strains of transgenic algae and their ideal growth conditions to increase the productivity of algal oil for the commercial success of industrial-scale biodiesel production. Besides increased product synthesis, genetic modifications at a minor level, such as the expression of certain proteins, responsible for cell aggregation during a specific growth stage, may also improve the rate of cell harvesting, which is one of the critical steps towards commercial success of algae-based biofuel production. Moreover, a genetic modification of algae seems to be promising and rather more successful towards biofuel production; however, transgenic algae may pose several environmental risks if exposed to ecosystems. Therefore, farming of transgenic algae must be under controlled conditions, preferably in a closed cultivation systems. Moreover, extensive research advancements in the development of bioreactors or producing genetically modified microorganisms may establish the economy of future biofuel production.

Overall, system development for the commercial success of algae based biofuel production to compete the fossil fuel is still a big challenge. Large scale production of algae is technically feasible, but it is not yet economically feasible. Research should be focused on reducing the overall costs while increasing algal biomass production and their lipid content. Several concerns regarding the mass cultivation of algae and their downstream processing must be addressed to advance the algae based biofuel industries from its existing state to commercial profitability.

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